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TREATMENT OF BACTERIAL MENINGITIS AND PREDICTION OF DISEASE OUTCOMES IN CHILDREN

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To Amos, Amelie and Astrid

ABSTRACT

Bacterial meningitis remains a significant cause of childhood morbidity and mortality, despite reductions to the global meningitis burden resulting from immunisations against *Haemophilus influenzae* type b (Hib) and *Streptococcus pneumoniae*. Meningitis poses a threat to child health especially in resource-limited settings, where mortality rates can reach up to 50%, even with the use of effective broad-spectrum antibiotics. The extent of the host's immune response associates with bacterial meningitis outcomes. Consequently, new treatment modalities have focused on controlling the initial inflammatory burst. This doctoral thesis project evaluated the use of a continuous antibiotic infusion, in contrast to conventional boluses, combined with paracetamol as a treatment for childhood bacterial meningitis. In addition, this research attempted to identify new prognostic markers in the cerebrospinal fluid (CSF) of children with meningitis and examined the impact of children's vitamin D status on disease outcomes.

The use of a continuous four-day cefotaxime infusion combined with oral paracetamol was evaluated in a prospective, randomised, double-blind parallel-group trial conducted at the Paediatric Hospital of Luanda in Angola between 2012 and 2017. The control intervention consisted of conventional cefotaxime boluses four times daily and an oral placebo, using mortality by day 7 from treatment initiation as the primary outcome. The prognostic role of matrix metalloproteinases (MMPs), myeloperoxidase (MPO) and the antimicrobial protein cathelicidin in the CSF and vitamin D levels in serum were analysed retrospectively using a cohort from Latin America of children with bacterial meningitis.

Our prospective clinical trial showed no benefit from using a continuous cefotaxime infusion combined with paracetamol as a treatment for childhood bacterial meningitis in Angola. By day 7, 61 of 187 (32.6%) children in the intervention group and 64 of 186 (34.4%) children in the control group died (absolute risk difference 1.8%, 95% confidence interval -7.8% to 11.4%). Similarly, no differences emerged between the study groups in terms of neurological sequelae.

In addition, the retrospective studies identified MMP-8 as a promising prognostic marker for bacterial meningitis: upon admission, a CSF MMP-8 level greater than the median value increased the odds of death 4.9-fold. The other analysed MMP, MPO and cathelicidin were also expressed in the CSF of children with bacterial meningitis, but did not predict disease outcomes to a similar extent. Furthermore, children's vitamin D status upon admission did not associate with survival.

In conclusion, the prognosis of childhood bacterial meningitis in Angola could not be improved by using a continuous cefotaxime infusion and oral paracetamol. Many of the children in our study were severely ill when presentating at hospital, likely contributing to the poor outcomes and warranting further attention. CSF MMP-8, however, presented as a potential prognostic marker for the disease.

TIIVISTELMÄ

Bakteeriperäinen aivokalvotulehdus eli bakteerimeningiitti on yhä maailmanlaajuisesti merkittävä lasten kuolleisuutta aiheuttava tauti, vaikka rokotukset *Haemophilus influenzae* tyyppi b ja *Streptococcus pneumoniae* -bakteereita vastaan ovatkin lähes hävittäneet taudin korkean tulotason maista. Köyhissä olosuhteissa jopa puolet bakteerimeningiittiin sairastuvista lapsista menehtyy hoidosta huolimatta taudin seurauksena. Huono ennuste vaikuttaisi osittain liittyvän potilaan liian voimakkaaseen immuunivasteeseen, ja taudin ennusteen parantamiseen tähtäävät hoidot ovat jo pitkään kohdistuneet nimenomaan liiallisen immuunireaktion hillitsemiseen. Tässä väitöskirjatyössä tutkittiin jatkuvan antibiootti-infuusion ja parasetamolin yhdistelmää lasten bakteerimeningiitin hoitona, etsittiin potilaiden aivo-selkäydinnesteestä uusia bakteerimeningiitin taudinkulkua ennustavia tekijöitä, sekä selvitettiin lasten immuunireaktioon vaikuttavan D-vitamiinipitoisuuden vaikutusta bakteerimeningiitin ennusteeseen.

Jatkuvan neljä päivää kestävän kefotaksiimiantibiootti-infuusion ja suun kautta annostellun parasetamolin yhdistelmää lasten bakteerimeningiitin hoitona tutkittiin Angolan pääkaupungissa Luandassa sijaitsevassa lastensairaalassa satunnaistetussa, kaksoissokkoutetussa ja lumekontrolloidussa asetelmassa vuosina 2012–2017. Interventioryhmä sai edellä mainitun hoidon, kun taas vertailuryhmä sai kefotaksiimia tavanomaisin boluksin neljästi vuorokaudessa sekä lumelääkettä suun kautta. Tutkimuksen ensisijaisena lopputulosmuuttujana oli kuolema ensimmäisen viikon aikana hoidon aloituksesta. Latinalaisessa Amerikassa aikaisemmin kerätystä potilasaineistosta selvitettiin tulehdusmerkkiaineiden, matriksin metalloproteinaasien (MMP), myeloperoksidaasin (MPO) ja katelisidiinin, pitoisuuksia aivo-selkäydinnesteessä ja määritettiin niiden ennustearvoa taudinkulkuun. Lisäksi tutkittiin veren D-vitamiinipitoisuuden yhteyttä bakteerimeningiitin ennusteeseen.

Angolassa tehdyn tutkimuksen perusteella jatkuva kefotaksiimi-infuusio yhdistettynä parasetamoliin ei parantanut lasten bakteerimeningiitin ennustetta. Viikon kohdalla 61/187 (32.6 %) interventioryhmän lapsista ja 64/186 (34.4 %) vertailuryhmän lapsista oli menehtynyt (absoluuttinen riskiero 1.8 %, 95 % luottamusväli -7.8 % – 11.4 %). Myöskään neurologisen vammautumisen suhteen ryhmien välille ei tullut merkitseviä eroja.

Latinalaisessa Amerikassa kerätyistä näytteistä tehdyissä tutkimuksissa havaittiin bakteerimeningiittiä sairastavien lasten aivo-selkäydinnesteen korkean MMP-8 -pitoisuuden lisäävän menehtymisen kerroinsuhdetta 4,9-kertaisesti. Myös toista matriksin metalloproteinaasia (MMP-9), myeloperoksidaasia sekä katelisidiiniä todettiin aivo-selkäydinnesteessä, mutta näiden molekyylien ennustearvo oli pienempi. Veren D-vitamiinipitoisuudella ei ollut yhteyttä lasten kuolleisuuteen.

Jatkuvan kefotaksiimi-infuusion ja parasetamolin yhdistelmä ei parantanut bakteerimeningiittiä sairastavien lasten ennustetta Angolassa. Merkittävä osa lapsista oli kriittisesti sairaita jo hoitoon tullessa, mikä osittain selittänee tutkimuksessa todettua korkeaa kuolleisuutta. Aivo-selkäydinnesteen MMP-8 vaikuttaisi lupaavalta ennustekijältä lasten bakteerimeningiitissä.

RESUMO

A meningite bacteriana continua a ser uma causa significativa de morbimortalidade infantil, embora a imunização contra Haemophilus influenzae tipo b e Streptococcus pneumoniae tenham reduzido o número de casos. A doença representa uma ameaça para a saúde infantil, especialmente em locais com recursos limitados, a onde a taxa de mortalidade pode chegar a 50%, apesar do uso de antibióticos eficazes de amplo espectro. Os resultados da meningite bacteriana está relacionada com a resposta imunitária do hospedeiro e, consequentemente, novas modalidades de tratamento concentraram-se no controle da exacerbação inflamatória inicial. Este projeto de tese de doutoramento avaliou o uso de uma infusão contínua de antibiótico, em contraste com os bolus convencionais, combinados com paracetamol para tratamento da meningite bacteriana infantil, o estudo evidenciou novos marcadores de prognósticos no líquido cefalorraquidiano (LCR) das crianças com meningite e examinou qual o impacto da vitamina D no desfecho da doença.

O uso de uma infusão contínua de cefotaxima por 4 dias combinada com paracetamol oral foi examinado num estudo prospectivo, randomizado, duplo-cego, em grupo paralelo, realizado no Hospital Pediátrico de Luanda em Angola entre 2012 e 2017. A intervenção consistiu na administração de cefotaxima em bolus e placebo oral, foi avaliada a mortalidade no 7º dia após o início do tratamento. O papel das metaloproteinases da matriz (MMPs), mieloperoxidase (MPO) e a proteína antimicrobiana catelicidina no LCR e os níveis de vitamina D no soro foram investigados retrospectivamente usando uma coorte de crianças com meningite bacteriana da América Latina em relação ao prognóstico.

O nosso estudo clínico prospectivo não mostrou benefício em usar uma infusão contínua de cefotaxima combinada com paracetamol como tratamento da meningite bacteriana infantil em Angola. No dia 7, das 61 de 187 (32,6%) crianças no grupo de intervenção e 64 de 186 (34,4%) crianças no grupo controle morreram (diferença de risco absoluta 1,8%, intervalo de confiança de 95% -7,8% a 11,4%). Da mesma forma, não surgiram diferenças entre os grupos de estudo em que concerna as sequelas neurológicas.

Os estudos retrospectivos identificaram a MMP-8 como um marcador de prognóstico promissor para a meningite bacteriana na admissão, um nível de MMP-8 no LCR acima da mediana aumenta as chances de morte em 4,9 vezes. As outras MMPs examinadas, MPO e a catelicidina também foram avaliadas no LCR das crianças com meningite bacteriana mas não previram os resultados da doença na mesma extensão que os anteriores. A quantidade serica de vitamina D na admissão não se associou à taxa de sobrevivência.

Em conclusão, o prognóstico da meningite bacteriana infantil em Angola não pode ser melhorado usando uma infusão contínua de cefotaxima e paracetamol oral. Muitas crianças no nosso estudo estavam gravemente doentes a chegada ao hospital, o que contribui para a grande mortalidade, logo merecem mais atenção. O LCR MMP-8 apresentou-se como um potencial marcador prognóstico da doença.

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I Savonius O, Rugemalira E, Roine I, Leite Cruzeiro M, Peltola H, Pelkonen T. Extended Continuous β-Lactam Infusion with Oral Acetaminophen in Childhood Bacterial Meningitis: A Randomized, Double-Blind Clinical Trial. Clin Infect Dis 2020 April 3. [Epub ahead of print]

II Savonius O, Roine I, Alassiri S, Tervahartiala T, Helve O, Fernández J, Peltola H, Sorsa T, Pelkonen T. The Potential Role of Matrix Metalloproteinases 8 and 9 and Myeloperoxidase in Predicting Outcomes of Bacterial Meningitis of Childhood. Mediators Inflamm 2019:7436932.

III Savonius O, Helve O, Roine I, Andersson S, Fernández J, Peltola H, Pelkonen T. Swiftly Decreasing Cerebrospinal Fluid Cathelicidin Concentration Predicts Improved Outcome in Childhood Bacterial Meningitis. J Clin Microbiol 2016;54(6):1648–1649.

IV Savonius O, Helve O, Roine I, Andersson S, Saukkoriipi A, González Mata A, Peltola H, Pelkonen T. Cerebrospinal Fluid Cathelicidin Correlates With the Bacterial Load and Outcomes in Childhood Bacterial Meningitis. Pediatr Infect Dis J 2018;37(2):182–185.

V Savonius O, Pelkonen T, Roine I, Viljakainen H, Andersson S, Fernandez J, Peltola H, Helve O. Vitamin D was not associated with survival or cerebrospinal fluid cathelicidin levels in children with bacterial meningitis. Acta Paediatr 2018;107(12):2131-2136.

The publications are referred to in the text by their Roman numerals. In addition, some unpublished data are presented.

ABBREVIATIONS

25-OHD	25-hydroxyvitamin D
CFR	case fatality rate
CI	confidence interval
CNS	central nervous system
CRAMP	cathelin-related anti-microbial peptide
CSF	cerebrospinal fluid
CT	computed tomography
E. coli	*Escherichia coli*
ELISA	enzyme-linked immunosorbent assay
GBS	group B streptococcus, *Streptococcus agalactiae*
GCS	Glasgow coma scale
GOS	Glasgow outcome scale
Hib	*Haemophilus influenzae* type b
H. influenzae	*Haemophilus influenzae*
HICs	high-income countries
ICP	intracranial pressure
ICU	intensive care unit
IgA	immunoglobulin A
IL	interleukin
IQR	interquartile range
LMICs	low- and middle-income countries
LPS	lipopolysaccharide
LTA	lipoteichoic acid
MIC	minimum inhibitory concentration
MMP	matrix metalloproteinase
MPO	myeloperoxidase
MRI	magnitic resonance imaging
MRSA	methicillin-resistant *Staphylococcus aureus*
MSSA	methicillin-susceptible *Staphylococcus aureus*
MyD88	myeloid differentiation factor 88
N. meningitidis	*Neisseria meningitidis*
OR	odds ratio
PAE	post-antibiotic effect
PAF	platelet-activating factor
PCR	polymerase chain reaction
PCV	pneumococcal conjugate vaccine
RNS	reactive nitrogen species
ROS	reactive oxygen species

SD	standard deviation
S. pneumoniae	*Streptococcus pneumoniae*
spp.	Lat. *species pluralis*, multiple species
TIMP	tissue inhibitor of metalloproteinase
TLR	Toll-like receptor
TNF	tumour necrosis factor
T>MIC	the time an antibiotic's concentration exceeds the MIC of a given pathogen at the site of infection
VDR	vitamin D receptor
WHO	World Health Organisation

1 INTRODUCTION

The human central nervous system (CNS) is surrounded by three membranes or meninges. These are called *pia mater*, *arachnoidea mater* and *dura mater*, anatomically ordered starting from the innermost membrane.[1] The first two of these together constitute the leptomeninges, which surround the subarachnoidal space filled with cerebrospinal fluid (CSF). Inflammation of the leptomeninges is called meningitis, and if bacterial in origin, the condition is referred to as bacterial meningitis.

Described as early as the fifth century BCE in *Corpus Hippocraticum*, bacterial meningitis is one of the most feared infectious diseases in history. The discovery of antibiotics at the beginning of the twentieth century dramatically improved disease prognosis, and the subsequent introduction of vaccines against common causative bacteria reduced meningitis incidence worldwide. Despite these advances, bacterial meningitis still exerts a heavy toll on childhood mortality and morbidity. Disease prognosis remains poor, especially in resource-limited settings, and few advances have been made in recent decades in the treatment of this severe disease.

With this in mind, this doctoral thesis evaluates a potential new treatment regimen for childhood bacterial meningitis and seeks to identify new markers capable of predicting disease prognosis.

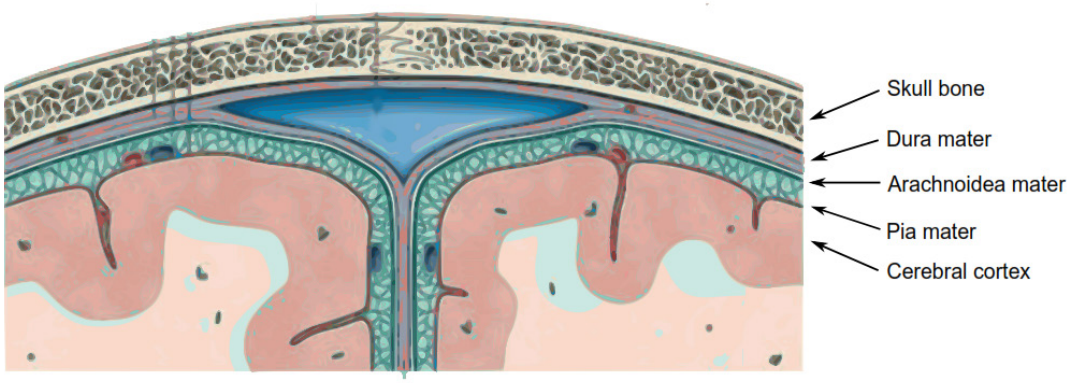

Figure 1 Anatomical model of the meninges. Modified from Drake *et al.*[1]

2 REVIEW OF THE LITERATURE

2.1 BACTERIAL MENINGITIS

2.1.1 Epidemiology

Bacterial meningitis remains a significant cause of childhood mortality and morbidity, representing the tenth most common cause of death in children under 5 years of age globally.[2] In 2016, meningitis accounted for an estimated 150 000 deaths in children under 5.[3] The epidemiology of childhood bacterial meningitis largely depends on both the age of the child and the child's country of origin. Thus, neonates, toddlers and older children from different parts of the world experience a largely varying disease incidence.[3-5] The definition of age groups also varies slightly; but typically, neonatal meningitis refers to meningitis in a child under the age of 2 to 3 months.

The incidence of bacterial meningitis peaks during the first months of life, oscillating at around 50 to 100 cases/100 000 children annually in the United States.[4] After the neonatal period, the incidence declines to 5 to 10 cases/100 000 in children under 2 years of age and further to roughly 0.5 annual cases/100 000 children in older age groups.[4] Similar trends in the age-related incidence have been reported in Finland as well as in England and Wales.[6,7] In 2019, a total of 20 cases of childhood bacterial meningitis, defined as a positive CSF bacterial culture in a child under 15 years of age, were reported to the National Infectious Diseases Register in Finland.[8]

The primary burden of bacterial meningitis falls, however, on children living in low- and middle-income countries (LMICs).[2] In sub-Saharan Africa, incidence varies significantly depending on the meningococcal epidemics, during which the incidence of meningococcal disease may reach up to 1000 cases/100 000 population annually.[9] Nonetheless, the annual incidence of *Haemophilus influenzae* type b (Hib) meningitis alone in Africa was previously estimated at between 50 to 100 cases/100 000 children under 5 years old.[10] This heavy burden of disease is at least partly explained by the lack of immunisation against Hib at the time of writing; in Malawi, for instance, the annual incidence of bacterial meningitis in non-neonatal children under 5 years old decreased from 154.4 to 20 per 100 000 following the introduction of this vaccine.[11]

However, using data from the 2017 Global Burden of Disease Study, the overall annual incidence of bacterial meningitis in sub-Saharan Africa in 2017 was estimated as reaching as high as 350 cases/100 000 children and young adults under 20 years of age.[13]

Although the 'meningitis belt' in Africa obviously represents the global hot spot of bacterial meningitis (Figure 2), certain regions in Asia also suffer from a substantially high burden of disease. A recent meta-analysis proposed a mean annual incidence of 105 cases of bacterial meningitis/100 000 children aged 1 to 59

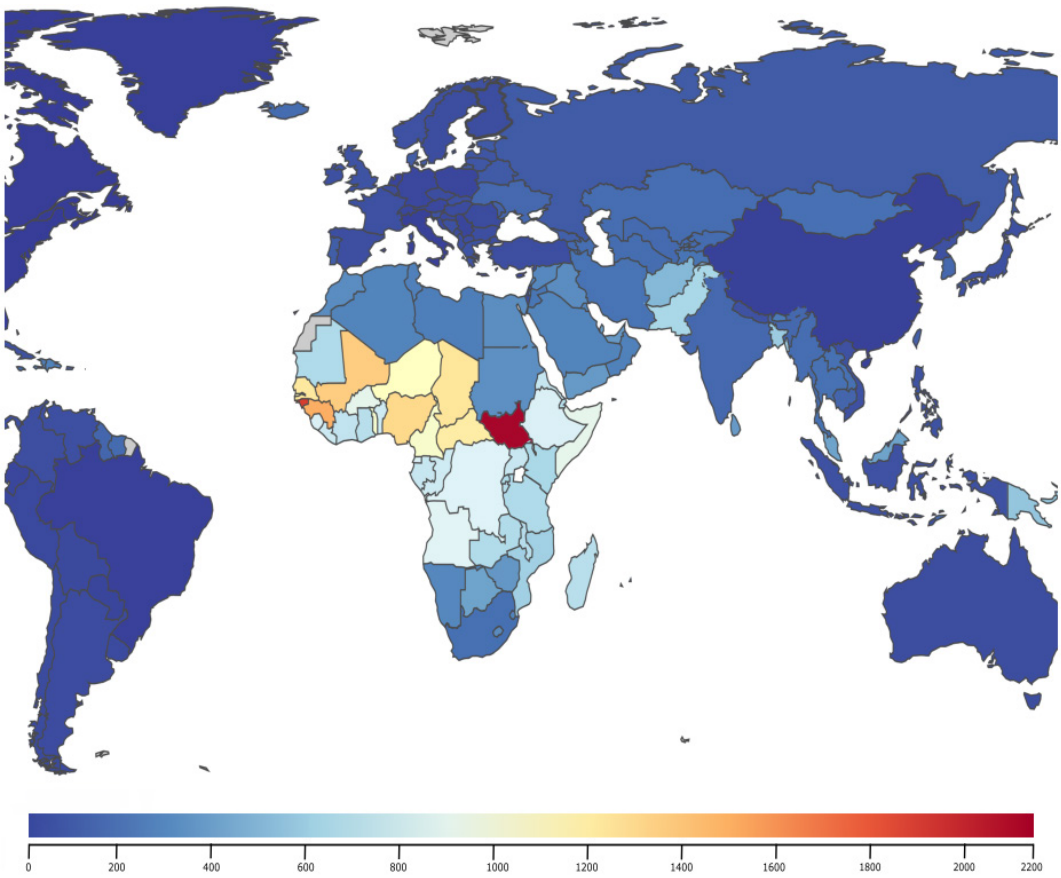

Figure 2 The estimated global incidence of meningitis in children under 5 years of age in 2017. The number of new cases per 100 000 children per year. Modified from the Institute for Health and Metrics (GBD Compare).[12]

months old in South Asia.[14] Furthermore, out of the 10 countries with the highest overall meningitis mortality in 2016, four were located in Asia (Afghanistan, China, India and Pakistan).[3]

2.1.2 Aetiology

Many bacteria can cause bacterial meningitis, although a relatively small group of pathogens account for the majority of cases in children. Before the introduction of conjugate vaccines, Hib, *Streptococcus pneumoniae* (*S. pneumoniae*) and *Neisseria meningitidis* (*N. meningitidis*) were the predominant bacteria causing childhood bacterial meningitis. However, this picture has shifted in recent decades. The aetiological distribution differs substantially between neonates and older children, and these are therefore discussed separately in the following sections.

2.1.2.1. Neonates

Group B streptococcus (*Streptococcus agalacticae*, GBS) and *Escherichia coli* (*E. coli*) are the predominant bacteria that cause neonatal meningitis in high-income

countries (HICs). Overall, GBS appears to account for up to 60% of all cases and *E. coli* for roughly 20%.[15-20] However, individual patient characteristics, such as gestational age and the child's age at admission, alter the aetiological distribution. Early-onset disease, commonly described as meningitis occurring during the first week of life, and late-onset disease, occurring after the first week, differ in terms of the bacterial aetiology. Specifically, GBS appears to explain a larger proportion of early-onset diseases, whilst the aetiology of late-onset disease is more diverse.[16,19] Moreover, preterm infants seem to be at higher risk for non-GBS meningitis, especially for early-onset disease.[16,19]

In addition to GBS and *E. coli*, bacteria that cause neonatal meningitis include other Gram-negative bacteria such as *Klebsiella, Enterobacter* and *Salmonella* spp.; *Listeria monocytogenes*; other Gram-positive cocci such as *Staphylococcus aureus* and coagulase-negative staphylococci, *Enterococcus* spp. and *Streptococcus* spp.; and, finally, *S. pneumoniae* and *N. meningitidis*, common aetiological agents of meningitis in older children.[15-17]

In LMICs, the spectrum of bacteria that cause neonatal meningitis is more diverse and less dominated by GBS.[21,22] Considerable heterogeneity has been reported in studies conducted in different geographical locations. In general, however, Gram-negative bacteria, such as *Salmonella* and *Klebsiella* spp., as well as *S. pneumoniae,* seem to play a more important role in these settings.[21,22]

2.1.2.2 Children beyond the neonatal period

The aetiology of non-neonatal childhood bacterial meningitis has undergone a major shift in recent decades, primarily due to the emergence of routine immunisations against Hib in many countries. In the prevaccination era, Hib was the leading cause of non-neonatal bacterial meningitis. Following the introduction of the conjugate Hib vaccine in the late 1980s, Hib meningitis has virtually disappeared in countries with adequate immunisation coverage.[23] Initially a privilege of HICs, the Hib conjugate vaccine is now a part of the routine immunisation schedule in most countries worldwide, with an estimated global coverage of 72%.[24]

However, as shown in Figure 3, vaccine coverage remains insufficient in many LMICs, where Hib meningitis remains a significant threat to child health.[25,26] Meningitis caused by Hib is primarily a disease of children aged 3 months to 3 years. Up to 90% of cases occur in children under 5 years of age, although the youngest infants are protected by maternal antibodies.[27,28]

Given routine immunisations against Hib, *S. pneumoniae* and *N. meningitidis* remain the two major bacteria causing childhood bacterial meningitis worldwide.[29] In order to prevent invasive diseases caused by *S. pneumoniae* in children, a heptavalent protein-polysaccharide conjugate vaccine (PCV7) was developed and licensed in the United States in 2000 and one year later in the European Union. Although not as striking as the effect of the Hib vaccine, a decline in paediatric pneumococcal meningitis cases was noted in many countries where PCV7 was included in the routine immunisation schedule.[4,30,31] Unfortunately, the emergence of pneumococcal serotypes not included in the heptavalent vaccine was registered following vaccine implementation,[32] leading to the replacement of PCV7 with either a 10-valent (PCV10) or a 13-valent vaccine (PCV13) in many countries in the late 2000s. Whilst a significant decrease in

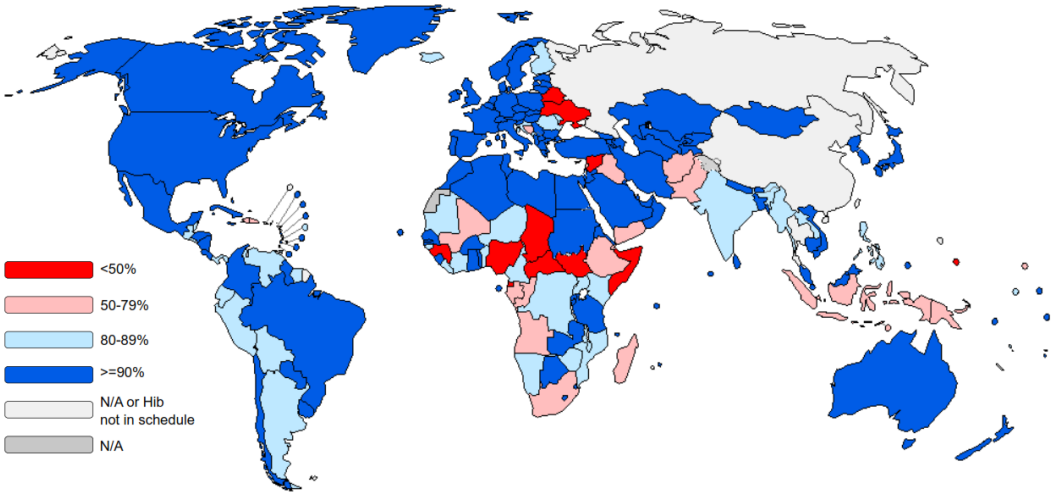

Figure 3 Global coverage of the Hib conjugate vaccine in 2017. Modified from World Health Organization (WHO) data.[24]

paediatric pneumococcal meningitis cases was registered shortly after the launch of PCV13, worrying signs of further serotype replacement have recently been reported.[33] The emerging serotypes vary by region: after PCV13 implementation, serotype 24F was responsible for most pneumococcal meningitis cases in France, whilst serotype 8 seemed to dominate in England and Wales.[33,34] Thus, novel vaccines targeting larger sets of pneumococcal serotypes are again being investigated. Viewed from a global perspective, the achievements of PCVs are yet to be achieved in many regions of the world. For example, a recent study suggested that four LMICs in Asia and Africa accounted for half of all pneumococcal deaths in HIV-uninfected children in 2015.[25] Childhood pneumococcal meningitis has the highest incidence in children under 2 years of age, and certain serotypes (such as serotype 1) are also capable of causing smaller epidemics.[28,35]

For *N. meningitidis*, the global picture is more complex due to the varied serogroup distribution in different parts of the world. In general, serogroups B and C account for most meningitis cases in the Americas and Europe, whilst serogroups A and C are predominant in Africa and Asia.[9] Moreover, serogroups W-135, Y and X also contribute to the meningococcal disease burden.

Within the 'meningitis belt' in sub-Saharan Africa, serogroup A has caused large epidemics for years reaching a devastating incidence of up to 1000 cases/100 000 population annually.[9,36] However, the interepidemic incidence also exceeds that of European countries manifold. A monovalent serogroup A conjugate vaccine (MenAfriVac) was licensed in 2009 and launched a year later, proving highly effective in reducing

the meningococcal meningitis burden following large-scale vaccination campaigns in those countries.[37] Given this success, further implementation of the vaccine into routine immunisation schedules is now ongoing, although some concern has arisen due to the emergence of other meningococcal serogroups in this region.[37,38]

Today in Finland, meningococcal meningitis primarily results from serogroup B, followed by serogroup C.[6] However, serogroup A has also previously caused epidemics in Finland, most recently in the 1970s.[39]

In the United States and within the European Union, a trend similar to Finland exists for invasive meningococcal disease.[40,41] Consequently, vaccines against serogroup C (and, later, against serogroup B) have been introduced in many European countries after the millennium shift, showing promising results.[28,40] The incidence of childhood meningococcal meningitis peaks during infancy, decreases thereafter and increases again in adolescence and young adulthood.[9]

In conclusion, the major global meningitis burden in non-neonates is today caused by *S. pneumoniae* and *N. meningitidis*, whilst GBS, *E. coli*, *S. pneumoniae* and other gram-negative bacteria comprise the most important pathogens for neonatal meningitis. In addition, Hib continues to cause disease in areas where vaccine-coverage remains suboptimal. As the availability of routine immunisations against the primary meningitis pathogens improves, further epidemiological shifts are likely. Such changes may remain unregistered if meningitis surveillance focuses only on the three primary pathogens, as is still common today. Indeed, this represents a significant challenge to future studies of the global childhood meningitis aetiology.

2.1.3 Predisposing factors

For neonatal meningitis, the main risk factors are similar to those for neonatal sepsis. These include perinatal and intrauterine infections such as endometritis or chorioamnionitis, rectovaginal colonisation of the mother with group B streptococci, prematurity or very low birth weight, invasive foetal monitoring and premature or a prolonged rupture of the membranes.[15,42] In addition, for late-onset disease, prolonged hospitalisation and the presence of external devices such as catheters increase the risk of infection.[42]

In older children, the conditions predisposing an individual to meningeal infection can roughly be divided into environmental and host-related risk factors. The strong seasonality of meningococcal meningitis in tropical settings serves as a striking example of the environmental impact. For instance, a review by Koutangni *et al.* estimated the incidence of endemic meningococcal meningitis to be 15-fold higher during the dry season compared to the wet season.[43] Although the underlying mechanism remains unclear, the hypothesis is that dry and dusty air facilitates bacterial invasion through the nasopharyngeal mucosa, possibly enhanced by preceding or co-existing viral infections.[43] Other environmental factors include crowded conditions, close contact with meningitis patients and recent mucosal colonisation with a pathogen that causes meningitis.[44]

The host-related predisposing conditions can further be divided into anatomical risk factors and immunodeficiencies. Anatomical risk factors comprise deficits in our anatomical barriers that aid the pathogen in entering the CSF, such as congenital anomalies (e.g., CSF fistula or breach), ventricular shunts, CNS trauma, cochlear implants and a preceding

neurosurgery.[27,44] The associated risk obviously varies according to the anatomy, but, for instance, children with cochlear implants were estimated to carry a more than 30-fold higher risk of pneumococcal meningitis compared to the overall incidence of the disease in children.[45] Furthermore, a nearby infection of the mastoids, sinuses or middle ear might result in a contiguous spread of bacteria into the CSF, thereby evading normal anatomical barriers.

A wide range of immunodeficiencies are associated with an increased risk of meningitis. Because bacterial meningitis is typically caused by encapsulated organisms, complement and antibody-mediated initial host defences play a crucial role. Thus, congenital complement and immunoglobulin deficiencies predispose individuals to disease.[46] A deficiency of properdin, a positive regulator of the alternative complement pathway, appears to increase the risk for meningococcal infections 250-fold compared to the overall disease incidence.[47] Anatomical or functional asplenia (commonly due to sickle cell disease) similarly increase the risk of bacterial meningitis: the odds ratio for pneumococcal and Hib meningitis in sickle cell disease patients in Africa was estimated as 25- and 9-fold higher, respectively, compared to controls.[48] Other immunodeficiencies detected in patients with bacterial meningitis include HIV, chemotherapy-associated immunosuppression and chronic illnesses such as congenital heart disease and liver disease.[49] Finally, certain demographic factors such as male gender, African American ethnicity and a low socioeconomic status associate with an increased risk of bacterial meningitis.[44]

2.1.4 Pathophysiology

The pathogenesis of bacterial meningitis in neonates is somewhat different from that in other age groups. In early-onset disease, the causative bacteria are often transmitted vertically from the mother, infecting the child either by direct transplacental transmission, via the amniotic fluid, or from the birth canal during delivery.[42,44] The infection of the meninges is predominantly a result of haematogenous seeding, in which the immaturity of the neonate immune system probably plays a significant role.[15,42,44] However, the following section will focus on the pathogenesis of bacterial meningitis after the neonatal period.

Our knowledge of the specific molecular mechanisms of the meningitis pathophysiology stems primarily from experimental studies, conducted either using cell cultures or based on different animal models of meningitis. Despite their limitations, such experimental studies provide insight into the underlying pathophysiology and have resulted in a deeper understanding of this complex disease.

2.1.4.1 Bacterial pathway: from initial presentation into the CNS

Less common routes for bacteria to reach the subarachnoidal space include the contiguous spread from a nearby infection (e.g., mastoiditis, or sinusitis), and direct entry into the CSF enabled by disruption of anatomical barriers (e.g., CSF shunts, congenital abnormalities, neurosurgery or trauma).[27,44,50] In childhood bacterial meningitis, however, the usual infection route results from the haematogenous spread from the nasopharyngeal mucosa, where prior colonisation occurred.[44,50]

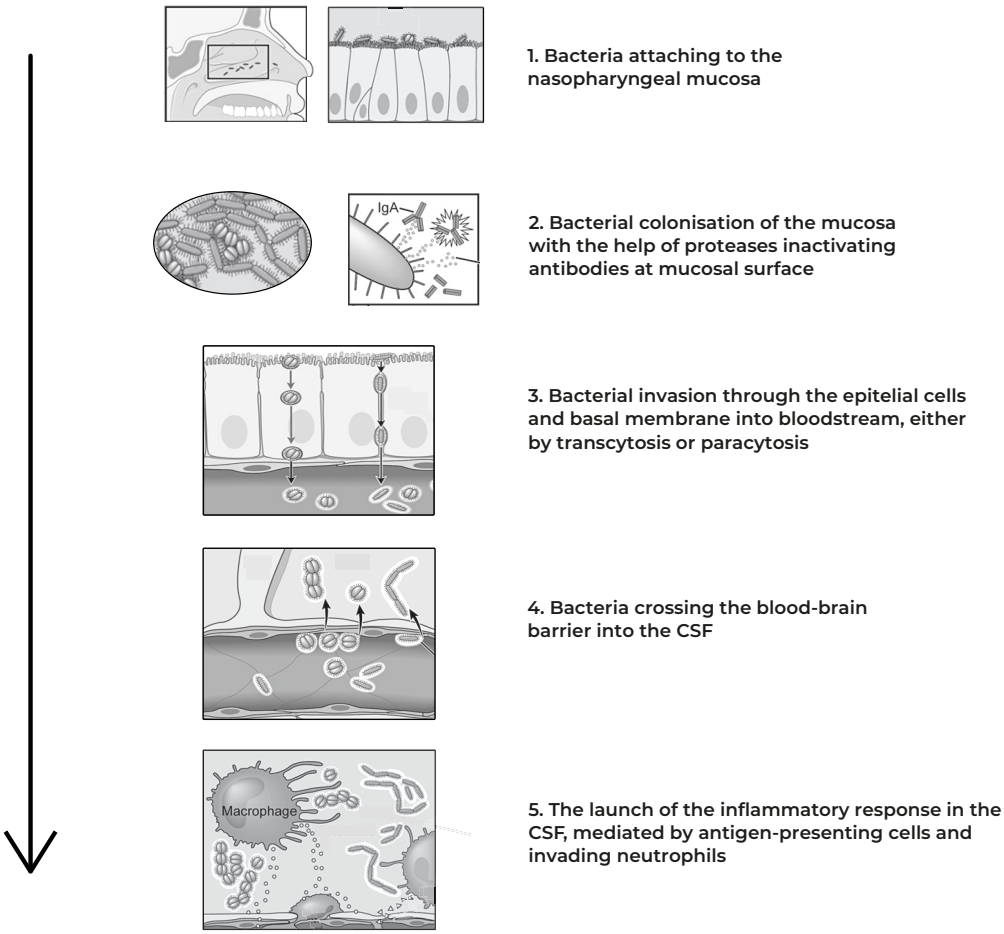

Figure 4 Bacterial pathway from the nasopharynx to the CSF. Modified from Mace.[44]

The pathway from the initial presentation of a bacterium in the nasopharynx to the invasion of the subarachnoidal space includes crossing several anatomical barriers and multiple exposures to the host's immune defence system, whilst the pathogens causing meningitis have evolved to cope with these challenges in different ways (Figure 4).

Typically, the first step of a bacterial infection leading to meningitis is the colonisation of the nasopharyngeal mucosa.[27] Important components of the mucosal immune defence, which invading bacteria must overcome, include the mechanical barrier of the mucus, the actions of the lysozyme and the secretion of immunoglobulin A (IgA). *S. pneumoniae*, *N. meningitidis* and Hib can synthesise IgA proteases, which cleave IgA and inhibit IgA-mediated opsonisation.[44,51,52] *S. pneumoniae* has been showed to express both enzymes that render the bacterium resistant to the lysozyme and enzymes decreasing the mucus viscosity, thereby facilitating colonisation.[51,52] In addition, the bacterium must be firmly attached to the mucosal epithelium; meningococci uses fimbria or pili to attach to the epithelial cells, whilst pneumococci makes use of phosphorylcholine and other binding proteins when attaching to the glycoconjugates or the platelet-activating fac-

tor (PAF) receptor expressed on the mucosal surface of the epithelium.[44,51,52]

The frequency of colonisation of the nasopharyngeal mucosa, or the asymptomatic 'carriage' of bacteria, differs across the three major meningitis pathogens. The prevalence of pneumococcal carriage peaks during the first 2 years of life, generally oscillating around 20% to 50% but reaching up to 100% in certain high-risk populations, and then typically begins to decline in subsequent years.[53] However, the serotypes causing disease often differ from those causing persistent nasopharyngeal colonisation, likely reflecting differences in invasiveness between pneumococcal serotypes.[28,35,53] Meningococcal carriage is less common, peaking in early adulthood with reported rates of around 20% in countries where serotypes B and C predominate.[54] In most of these individuals, infection is cleared by the immune system after a varying period of colonisation, whilst only a small fraction of cases develop invasive disease; also the strain-specific characteristics seem to play a role here.[54,55] For Hib, carriage rates in children generally peak at the age of 3 to 5 years, varying from 3% to over 10%.[28,56,57]

Whilst all of these pathogens seem to colonise the nasopharyngeal mucosa frequently, only a fraction of these colonisations lead to invasive disease. The subsequent step towards symptomatic infection include bacterial invasion through the epithelial cells and the underlying basal membrane into the bloodstream. Pneumococci appear capable of exploiting our endogenous transportation system of antibodies or the PAF receptor across the epithelial cells, and travel through the mucosa via transcytosis.[51,52] Meningococci seems to cross the epithelial barrier in a similar transcytotic way, whilst Hib separates the tight junctions and enters via paracytosis.[44] To further penetrate the basal membrane and the adjacent extracellular matrix, pneumococci may use hyaluronan lyase in order to degrade hyaluronan and certain chondroitins.[51,52]

In the bloodstream, bacteria are exposed to multiple host defence mechanisms, primarily complement-mediated opsonisation and phagocytosis. The polysaccharide capsule, which all three of the primary meningitis pathogens possess, seem to be of pivotal importance in avoiding the immune response, since it inhibits both phagocytosis and immunoglobulin- and complement-mediated bacterial destruction.[51,52] In addition, the pneumococcus expresses many proteins (e.g., pneumolysin, PspA and PspC) protecting it from the actions of the complement system and, thus, contributing to its virulence.[51,52] To further gain entry into the CNS, bacteria must cross either the blood–brain barrier (formed by cerebromicrovascular endothelial cells) or the blood–CSF barrier at the choroid plexuses (formed by epithelial-like cells). The pneumococcus seems to cross the blood–brain barrier via transcytosis, making use of the PAF receptor in the endothelial cells or possibly the laminin receptor.[51,52] For Hib, some data suggest that crossing the blood–CSF barrier at the choroid plexuses might be the preferred route of entry.[52]

2.1.4.2 The CNS immune response

The CSF constitutes an immunocompromised space within the human body, where complement molecules, immunoglobulins and polymorphonuclear leukocytes are largely absent under normal circumstances. Therefore, after gaining entry into the CSF, bacteria can effectively multiply. Ultimately, bacteria are identified by antigen-presenting

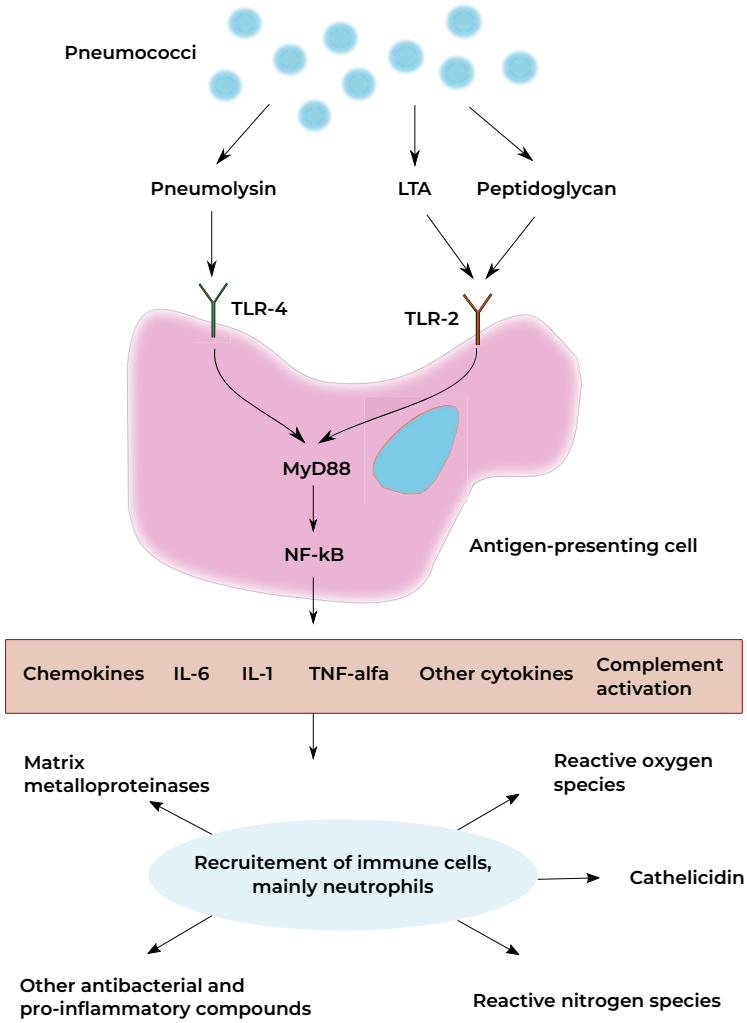

Figure 5 The launch of the inflammatory cascade in the CSF by pneumococci. Abbreviations: IL, interleukin; LTA, lipotechoic acid; MyD88, myeloid differentiation factor 88; NF-κβ, nuclear factor -κβ; TLR, Toll-like receptor; TNF-alpha, tumour necrosis factor –alpha. Modified from a review by Mook-Kanamori *et al.*[51]

cells such as the microglia with the help of pattern recognition receptors, leading to the launch of the local immune response. This launch of the immune response is promoted by bacterial autolysis, aggravated by exposure to bacteriolytic antibiotics and results in the release of pro-inflammatory bacterial compounds.[58] Selected parts of this cascade are illustrated in Figure 5.

The primary meningeal pathogens differ in terms of the expression of immunogenic molecules: for pneumococci, the primary inducers of the inflammatory host response include the subcapsular lipoteichoic acid (LTA), peptidoglycan, pneumolysin and bacterial DNA.[51,52] These immunogenic molecules are detected differently by resident immune cells. LTA and peptidoglycan seem to interact with Toll-like receptor (TLR)-2 via CD14, pneumolysin with TLR-4 and bacterial DNA with TLR-9 and possibly with different nod-like receptors; also

other pattern recognition receptors might play a role.[51] Overall, this detection of bacteria results in intracellular downstream molecular signalling, often including myeloid differentiation factor 88 (MyD88), leading to the nuclear translocation of transcription factor NF-κβ and the subsequent production of inflammatory cytokines and chemokines. For meningococci, endotoxin or lipo(oligo) polysaccharide (LPS) constitutes the major immunogenic molecule, interacting with TLR-4; however, TLR-2, TLR-9 and nod-like receptors also seem to contribute to this process.[55] Downstream signalling somewhat similar to pneumococcal infections has been reported in studies with meningococci.[55] Data on the molecular details of the CNS inflammatory response due to Hib meningitis remain scarce, but LPS released from bacteria seems to elicit strong inflammatory changes in the CSF, similar to meningococcal meningitis.[59]

The three major early-response cytokines, interleukin (IL)-1β, tumour necrosis factor alpha (TNF-α) and IL-6, are important in the CSF inflammatory response to bacterial meningitis. These cytokines are produced by several different cell types in the CNS (e.g., astrocytes, microglia and endothelial cells) upon bacterial invasion, and elevated levels have been noted in the CSF of patients with bacterial meningitis.[52,60] While TNF-α and IL-1β are mainly pro-inflammatory, IL-6 also exerts anti-inflammatory effects. The expression of inflammatory cytokines parallels the secretion of multiple chemokines as well as the production of complement components. Fundamentally, this leads to the upregulation of leukocyte migration adhesion molecules in the cerebral vasculature, and finally to the recruitment of circulating leukocytes (mainly neutrophils) through the blood–brain barrier into the CSF.[51,52]

Once at the site of infection, the immune cells begin combatting the bacterial invasion by different means. One important component of the initial immune response is the increased production of reactive oxygen species (ROS), which, by nature, are cytotoxic and effectively kill bacteria.[61] The enhanced production of ROS, also called the respiratory burst, is mediated by several oxidative enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and myeloperoxidase (MPO). Reactive nitrogen species (RNS) constitute another entity of oxidants used by the host immune cells to fight invading bacteria. Together, the production of RNS and ROS leads to the formation of the very strong oxidant peroxynitrite.[51,52]

Besides the above-mentioned oxidants, immune cells possess several other effector molecules in their arsenal. Antimicrobial peptides, such as defensins and cathelicidin (LL-37), are secreted mainly by phagocytic cells as part of the innate immune response.[62,63] Certain matrix metalloproteinases (MMPs) are also strongly expressed by the immune cells in the CSF in response to infection. Although discussed below more in detail, MMPs appear to play a role in degrading the blood–brain barrier and enhancing the local inflammatory cascade by processing cytokines and chemokines.[51] MMPs can, in turn, be activated by oxidants, further leading to aggravation of the inflammation.[64,65]

2.1.4.3 Neuronal damage

A substantial body of evidence supports our current understanding that pathophysiological events leading to neuronal damage during bacterial meningitis strongly relate to the host's immune response within the CNS.[50] In experimental meningitis, an excessive neu-

trophil function relates to a more severe disease and worse intracranial complications, and, consistently, inducing neutrophil apoptosis reduces the extent of complications and improves recovery.[66] The intracisternal injection of TNF-α or IL-1β results in CSF pleocytosis and elevated CSF protein and lactate levels.[67] CSF concentrations of TNF-α in patients with bacterial meningitis have been associated with disease severity,[68] and accordingly, TNF-α seems capable of directly mediating neuronal cell toxicity.[69] The experimental inhibition of inflammatory mediators such as cytokines, chemokines, leukocyte migration adhesion molecules and complement components have often resulted in the attenuation of the CSF inflammatory response and occasionally in reducing the meningitis-associated pathology.[51,52] However, inhibiting central pro-inflammatory pathways is not without cost, since the immune response consequently deteriorates: for instance, TNF-α knockout mice showed a higher mortality compared to wild-type mice in experimental meningitis,[70] and C3-deficient mice died earlier than wild-type controls, despite an attenuation of the blood–brain barrier disruption and lower intracranial pressure (ICP).[71] Thus, whilst the excessive immune response in meningitis seems partly responsible for the pathology, a too rigorous inhibition of it also results in poor outcomes, since direct bacterial cytotoxicity obviously contributes to parenchymal damage as well.[50]

Much research has been conducted on the downstream mediators of parenchymal injury in bacterial meningitis, primarily focusing on the above-mentioned oxygen- and nitrogen-centred oxidants and the family of MMPs. The latter will be discussed in more detail in Chapter 2.3.

In normal circumstances, ROS are produced at low rates as part of our basal metabolism. The cytotoxic effects of these compounds are balanced by different antioxidants such as glutathione, ascorbic acid and superoxide dismutase. During bacterial meningitis, the production of oxidants exceeds the neutralising capacity of the antioxidant system, resulting in the accumulation of ROS, local pro-inflammatory effects such as the activation of MMPs and, finally, in cellular damage.[61] Most of the detailed knowledge has again been derived from animal models of bacterial meningitis. In an infant rat model of streptococcal meningitis, the production of superoxide was detected in the brain of infected animals but not in healthy controls.[72] Furthermore, superoxide could not be detected in those animals which received a radical scavenger as adjuvant treatment, and meningitis-associated neuronal damage in the cortex and hippocampus was subsequently prevented.[72] In line with this result, another study suggested that the intraperitoneal administration of the antioxidant N-acetyl-l-cysteine reduced the CSF leukocyte influx and the increase in ICP and brain water content in experimental meningitis in rats.[73] Although some additional promising results exist, a recent study showed only modest beneficial effects from adjuvant N-acetyl-l-cysteine in a mouse model of pneumococcal meningitis, calling to question its potential as a future adjuvant treatment option.[74]

In humans, most oxidant studies have focused on the role of RNS in the pathophysiology of meningitis. Elevated levels of nitrite and nitrate, reflecting the production of nitric oxide, were detected in the CSF of patients with bacterial meningitis when compared to controls without CNS infection.[75] Similarly, bacterial meningitis resulted in an elevated CSF concentration of nitrotyrosine, a marker for the formation of the RNS per-

oxynitrite, and high levels of nitrotyrosine appeared to associate with an unfavourable outcome.[76] The same study identified nitrotyrosine residues in the subarachnoid space and the leptomeninges and in the leptomeningeal, cortical and some deep parenchymal blood vessels in a small autopsy series of patients who succumbed to pneumococcal meningitis. Animal studies have similarly showed elevated levels of RNS during bacterial meningitis, but experimental treatment with NO synthase inhibitors have yielded inconclusive results.[52]

The mechanisms by which these oxidants cause harm vary. Briefly, based on experimental studies, ROS and RNS seem capable of 1) inducing a disruption to the blood–brain barrier, 2) altering the cerebrovascular autoregulation in the CNS, 3) enhancing the inflammatory cascade locally and 4) causing direct neuronal damage.[77]

In conclusion, although intended to combat an infection, the immune response results in an accumulation of harmful toxins in the CSF, an increase in the blood–brain barrier permeability, and subsequent cerebral oedema. Together with direct bacterial toxicity, these pathological events lead to reduced cerebral perfusion, cerebrovascular complications and finally parenchymal damage.[44,51,61]

The histopathological findings of bacterial meningitis include cortical necrosis, hippocampal cell death, damage of the hair cells in the cochlea and damage of neurons in the spiral ganglion.[51,52,78] Although the exact pathophysiology of hearing impairment due to bacterial meningitis remains disputed, cochlear damage seems to associate with the inflammatory changes in the CSF and possibly result from labyrinthitis and the subsequent production of cytotoxic substances.[78,79] The cochlear aqueduct, connecting the subarachnoidal space to the scala tympani, might be the route for infectious spread into the inner ear.[79]

2.1.5 Clinical presentation

The clinical presentation of a child with bacterial meningitis is highly variable, depending upon the patient age and the stage of illness. The classic symptoms of bacterial meningitis are rarely seen in neonates and small infants, who often present with nonspecific symptoms such as irritability or lethargy, fever or hypothermia and a poor appetite.[15,22,42] The diagnosis in small infants requires a high level of suspicion, since these symptoms can be difficult to distinguish from those of sepsis. Meningitic signs in this age group include a bulging fontanel and possibly nuchal rigidity, but these are inconsistently found and might occur late during the course of disease. Depending on the geographic location, seizures have been reported in up to 50% of neonatal cases upon presentation.[15,18]

Beyond the neonatal period, common nonspecific symptoms at admission include fever, headache, nausea or vomiting, photophobia, irritability or lethargy and an impaired consciousness.[80] Signs of meningeal inflammation, such as neck stiffness or the Kernig and Brudzinski signs, are more easily identifiable in this age group, and carry a rather solid predictive value for bacterial meningitis. For instance, in a cohort of children admitted to hospital for suspected meningitis, 39% of those with any sign of meningeal irritation had bacterial meningitis, when assessed by a paediatrician.[81] A retrospective study of 108 children with bacterial meningitis in Finland showed, however, that nuchal rigidity was detected in 4 out of 5 children aged 12 months

or older, and in only 2 out of 3 children with short-duration (<2 days) symptoms.[82] Thus, the absence of nuchal rigidity does not rule out bacterial meningitis in older children.[18]

When the disease progresses, seizures and focal neurological signs become more common. In Angola, where patients (median age 14 months) presented at hospital after a median of 5 days of illness, 70% had seizures prior to hospital admission, and 1 in 4 children showed focal neurological signs.[83] Typical focal neurological findings in bacterial meningitis include cranial nerve deficits or mono-, hemi- or quadriparesis. Such findings might reflect the development of cerebrovascular complications.[84,85]

Finally, some symptoms seem to associate with specific bacterial agents. Petechiae or purpurae most commonly accompany systemic meningococcal disease, although they possibly occur with other causative agents as well. Waterhouse–Friderichsen syndrome, characterised by widespread purpurae associated with severe shock and adrenal gland failure, is similarly often the result of meningococcemia. Yet, joint involvement should lead clinicians to lean towards meningococcal or Hib infection.[27]

Figure 6 Child suffering from bacterial meningitis in Angola. The extended extremities, the pronation of the hands and the arched back are signs of opisthotonus, reflecting a complicated disease. Printed with the family's permission. Photo credit: Author's own, 2017.

2.1.6 Diagnosis and differentials

The diagnosis of bacterial meningitis relies on the examination of the CSF, aiming to detect bacteria and signs of meningeal inflammation. The CSF is obtained by performing a lumbar puncture, whereby a small caliber needle is inserted between the lumbar vertebrae into the subarachnoidal space, from whence the CSF can be collected.

Previously, guidelines have recommended performing a computed tomography (CT) scan of the head prior to a lumbar puncture in order to detect signs of elevated ICP or a possible brain mass, which might increase the risk of brainstem herniation following the puncture. This approach is, however, debatable, since a normal head CT scan does not exclude the possibility of herniation, and on the other hand, ICP increases to some extent in most meningitis cases without causing herniation.[86] Currently, most guidelines recommend performing

a thorough clinical examination before a lumbar puncture, and if no signs or symptoms suggest the presence of a raised ICP or intracranial masses, a lumbar puncture without any prior imaging is recommended when bacterial meningitis is suspected.[18,87,88] When neuroimaging is considered necessary and resources permit, magnetic resonance imaging (MRI) is an attractive alternative to CT, since MRI provides more information concerning possible differential diagnoses such as viral CNS infections.[89] Overall, however, herniation is a rare complication of bacterial meningitis, and performing a lumbar puncture is safe in most patients.[86]

The presence of bacteria in the CSF can be directly detected with a Gram stain, the yield of which depends on the CSF bacterial counts as well as on the causative pathogen.[80] A bacterial culture of the CSF represents 'the gold standard' of meningitis diagnostics, and reveals, when positive, the definitive organism and its antibiotic susceptibility. The yield of the CSF bacterial culture obviously decreases if empiric antibiotics have been administered before the lumbar puncture, which is often the case especially in resourced-limited settings.[80] A study by Kanegaye *et al.* suggested that the sterilisation of the CSF occurs within 2 hours of parenteral antibiotic treatment initiation in meningococcal infections and after 4 hours in pneumococcal infections, highlighting the utility of pretreatment lumbar punctures.[90]

The presence of meningeal inflammation can be estimated by measuring the number of white blood cells and the protein and glucose levels in the CSF. The typical CSF finding in bacterial meningitis comprises a CSF pleocytosis (commonly 1000–5000 cells/mm^3) with predominantly polymorphonuclear leukocytes, a reduced CSF glucose concentration (CSF glucose <0.6–2.5 mmol/L or <10–45 mg/dL) and an elevated level of CSF protein (>100 mg/dL).[80,88] These parameters should be interpreted together, given that irregularities such as normal CSF white cell counts and lymphocyte predominancy early in disease may occur.[91] Indeed, according to WHO guidelines, even the presence of >100 leukocytes/mm^3 in the CSF of a child with compatible symptoms indicates the presence of bacterial meningitis.[92] The interpretation of the CSF parameters is particularly difficult in neonates, in whom both the white cell count as well as the glucose and protein levels might overlap between healthy individuals and infants with bacterial meningitis.[18,42] These parameters change during the first months of life, and healthy neonates present with higher CSF white cell counts and protein levels, but slightly lower glucose levels compared to infants older than 28 days.[93]

In patients who have received antibiotics before a lumbar puncture is performed in particular, nonculture diagnostic methods might provide additional assistance to the clinician. Latex agglutination is a simple and rapid method in which antibodies directed against the capsular polysaccharides of the meningeal pathogens are used for bacterial detection. Whilst not requiring any special equipment, latex agglutination tests can be easily used in resource-limited settings, although the routine use of these tests does not seem to provide an additional benefit partly due to their poor sensitivity.[18,88]

Several new diagnostic tools have been developed in recent decades which identify bacteria in the CSF. Broad-range polymerase chain reaction (PCR) assays targeting the 16S region of bacterial ribosomal RNA seem to demonstrate a high sensitivity and specificity, although differences exist in the performance of the assays.[94] Similarly,

the commercially available PCR-based Filmarray® meningitis/encephalitis panel seems to perform well in the diagnosis of bacterial meningitis.[95] Overall, PCR-based methods are probably most useful in cases where antibiotic treatment has been initiated before a lumbar puncture, since the diagnostic sensitivity remains unaffected by the antibiotic activity in the CSF.[96] A completely novel approach to the diagnostics of CNS infections relies on the use of metagenomic sequencing, where all genetic information in the CSF is sampled and the data are then analysed using powerful bioinformatic tools to reveal possible infectious agents.[97]

Although different PCR-based assays are today widely used in HICs together with conventional diagnostics, the equipment required for these analytics often limit their applicability in resource-limited contexts.[98] Indeed, the WHO recently highlighted the need for rapid diagnostic tests suitable for low-resource settings in their 'Defeating meningitis by 2030' roadmap.[99]

After the above-mentioned examination of the CSF and possibly the use of nonspecific inflammatory markers such as C-reactive protein and procalcitonin, the clinician must consider possible differential diagnoses. Depending upon the presentation and the local endemic situation, these include a large variety of diseases. If signs of meningeal irritation are present, the differentials include alternative causes of meningitis: viral, tuberculous, fungal, carcinomatous, drug-induced and meningitis associated with systemic inflammatory diseases such as sarcoidosis.[98] The probability of many of these depends on the medical history of the patient and, in particular, immunosuppression of any kind is relevant, since it predisposes an individual to more uncommon causes of meningitis. Without predisposing factors, viral meningitis likely remains the most common diagnosis.[98] Moreover, subarachnoidal haemorrhage might cause reactive meningeal irritation.

In the absence of meningeal irritation, the list of differentials expands significantly since both many infectious and noninfectious diseases might cause the above-mentioned nonspecific symptoms of meningitis including fever, vomiting, headache, irritability or impaired consciousness. The decision to undertake or withhold a lumbar puncture in these patients is crucial, and generally, a lumbar puncture should be performed if any suspicion of meningitis exists (and no contraindications are present).[87]

In tropical regions, cerebral malaria is an important differential diagnosis of meningitis. The diagnosis of cerebral malaria remains disputed, traditionally defined as *P. falciparum* parasitaemia with a clinical presentation of unarousable coma following exclusion of other possible reasons for the coma. This definition might lead to overdiagnosing cerebral malaria, given problems of microbiological diagnostics in resource-limited settings and the occurrence of asymptomatic parasitaemia in malaria-endemic areas. Consequently, the presence of malarial retinopathy is today commonly included in the diagnostic criteria.[100] In general, bacterial meningitis should always be considered, even though a febrile comatose child presents with malaria parasitaemia.[101]

2.1.7 Treatment

At the beginning of the twentieth century, virtually all patients with pneumococcal or Hib meningitis and most of those with meningococcal meningitis succumbed to the disease.[102] The use of specific antiserums,

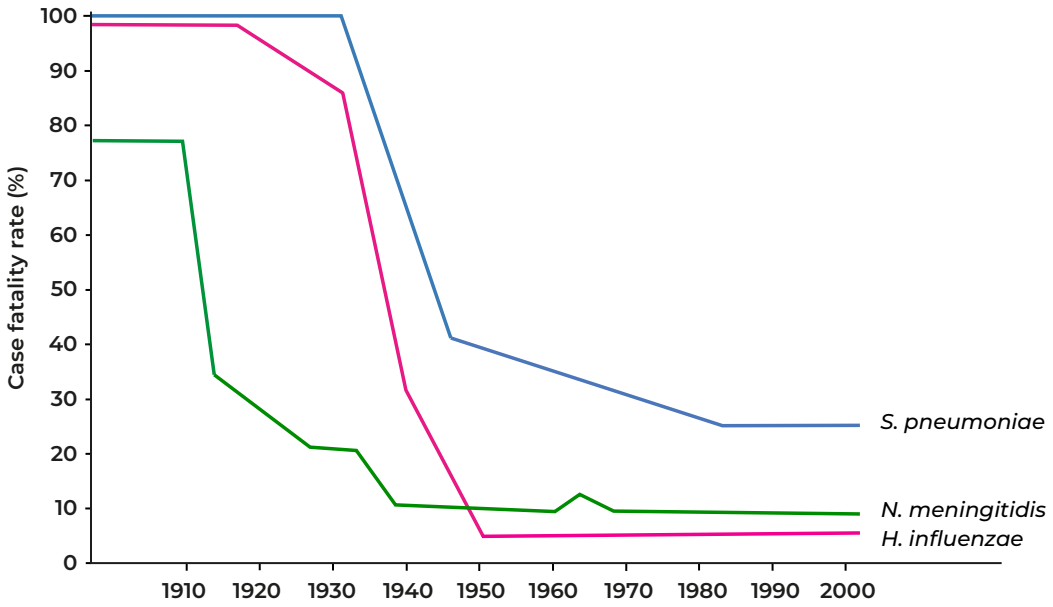

Figure 7 Case fatality rates for bacterial meningitis during the last century. Modified from Swartz.[102]

first delivered intrathecally as treatment of meningococcal meningitis in 1906, reduced the mortality of meningococcal meningitis to 30.9% and later the mortality of Hib meningitis to roughly 85%.[102,103] The major breakthrough, however, was the introduction of antibiotics such as sulfonamides, chloramphenicol and penicillin in the treatment of bacterial meningitis after the 1920s. This resulted in a striking improvement to the prognosis of meningococcal, pneumococcal and Hib meningitis (Figure 7).[102]

Since then, much research has been conducted on the optimal treatment of bacterial meningitis and new antibiotics have emerged, but further major improvements to disease outcomes remain unachieved (Figure 7). The following section summarises present recommendations for the antibiotic treatment, adjuvant treatment and supportive treatment of childhood bacterial meningitis.

2.1.7.1 Antibiotic treatment

The empiric antibiotic treatment of bacterial meningitis aims to cover the most likely causative organisms and consequently depends on the child's age. In children beyond the neonatal age (>1–3 months of age, depending on the definition used), a third-generation cephalosporin such as cefotaxime or ceftriaxone remains the drug of choice.[18,27,80,104,105] The addition of vancomycin or rifampicin should be considered if pneumococcal resistance to cephalosporins is prevalent,[106] although conclusive clinical data on the benefit of this combination is lacking.[18] In low-resource settings, the WHO recommends the use of a third-generation cephalosporin, or chloramphenicol combined with ampicillin or benzylpenicillin as empiric treatment.[92] However, the latter combination is suboptimal, since both Hib and nontyphoidal salmo-

nellae are often resistant to chloramphenicol and ampicillin.[107]

The aetiological distribution is somewhat broader in neonates compared to older infants, including more Gram-negative bacteria as well as agents intrinsically resistant to cephalosporins such as the *Enterococcus* spp. and *L. monocytogenes*. Thus, the recommendations for the empiric treatment of neonatal meningitis usually include ampicillin combined with cefotaxime or, alternatively, ampicillin combined with an aminoglycoside.[18,27,80,104,106] Ceftriaxone is often avoided in neonates since it may cause biliary sludging and hyperbilirubinemia, and if mixed with calcium-containing solutions, result in ceftriaxone-calcium precipitates. If pneumococcal meningitis is suspected and strains with reduced susceptibility are prevalent, the addition of vancomycin might also be considered. In low-resource settings, the WHO primarily recommends ampicillin combined with gentamycin.[92]

Provided that the CSF culture yields an aetiological agent and antibiotic susceptibilities are obtained, antibiotic treatment should thereafter be targeted. Table 1 provides the recommended antibiotic treatment for the most common aetiological agents of bacterial meningitis in neonates and older children.

The recommended duration of antibiotic therapy traditionally oscillates around 7 days for meningococcal or Hib meningitis and 10 to 14 days for pneumococcal meningitis.[106] In low-resource settings, the WHO recommends antibiotic treatment for 7 to 10 days in non-neonates.[92] However, shorter courses of antibiotic treatment would likely prove legitimate in children recovering well: a large multicountry double-blind equivalence study showed no difference in treatment failures between 5 versus 10 days of ceftriaxone treatment in meningitis primarily caused by

Table 1. Pathogen-specific recommendations for the treatment of childhood bacterial meningitis

Pathogen	Antibiotic	Ref.
S. pneumoniae (penicillin-susceptible)	Penicillin G, ampicillin	18,27,106
S. pneumoniae (penicillin-resistant)	Third-generation cephalosporin ± vancomycin	18,27,104,106
N. meningitidis	Penicillin G, ampicillin / third-generation cephalosporin*	18,27,104,106
H. influenzae	Third-generation cephalosporin	18,27,104,106
GBS	Penicillin, ampicillin / third-generation cephalosporin ± gentamycin	27,104,106
E. coli	Third-generation cephalosporin / meropenem*	27,106
S. aureus (MSSA)	Flucloxacillin, oxacillin, nafcillin	18,106
S. aureus (MRSA)	Vancomycin	18,106
L. monocytogenes	Ampicillin + gentamycin	18,27,104,106

*The latter choice should be based on local resistance patterns. Abbreviations: MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-sensitive *S. aureus*.

Hib, *N. meningitidis* or *S. pneumoniae*.[108] Moreover, during meningococcal epidemics in sub-Saharan Africa, one or two doses of intramuscular chloramphenicol or ceftriaxone are commonly used and appear to yield comparable and reasonable outcomes.[109] In neonates, the recommended treatment duration is usually longer, ranging from 10 to 14 days in GBS meningitis to a minimum of 3 weeks for infection from *E. coli*.[27,42,104] Finally, meningitis caused by nontyphoidal salmonellae constitutes a special entity due to the intracellular survival of the pathogen. Consequently, recommendations outline treatment lasting for a minimum of 4 to 6 weeks.[110]

Given that the appropriate empirical antibiotic is chosen, prompt initiation of antimicrobial therapy is often emphasised. Several adult studies have pointed to a benefit from early initiation of antibiotic treatment, but, for understandable reasons, no randomised prospective trials on this exist.[88,111] A guideline revision in Sweden in 2009, encouraging a prompt lumbar puncture and the initiation of antibiotic treatment without a preceding CT, led to better disease outcomes in adults with bacterial meningitis: this study suggested a 12.6% relative increase in mortality per hour of in-hospital treatment delay.[112] Furthermore, a prospective observational study of adult patients in the intensive care unit (ICU) with pneumococcal meningitis suggested that an in-hospital delay in antibiotic treatment initiation of more than 3 hours increased the odds of death 14-fold.[113] Some experts suggest that the key factor is initiating treatment before a patient's condition deteriorates, 'before the therapeutic window closes'.[88,114] In conclusion, adequate treatment should be initiated as soon as possible when bacterial meningitis is suspected.[88]

2.1.7.2 Adjuvant treatment

The adjuvant treatment of bacterial meningitis remains contested, although a plethora of both clinical and experimental studies have been conducted to address this issue. Numerous candidate adjuvant therapies have shown promising results in animal studies, but only a few have thus far been evaluated in randomised clinical trials in children.

Dexamethasone

Dexamethasone is currently the only adjuvant treatment in clinical use, broadly recommended for children and adults with bacterial meningitis in HICs.[18,87,115] The rationale for using dexamethasone originates from studies pointing towards the harmful effects of the initial immune response (see discussion above in Chapter 2.1.4.3). Adjuvant dexamethasone treatment has demonstrated both harmful and beneficial effects in animal studies. Yet, whilst these studies have been conducted using different animals and experimental designs, the overall effect has not been clarified by these investigations.[60,114,116]

The results from clinical trials in children are similarly heterogeneous. Based on older studies, dexamethasone seemed to reduce the incidence of hearing sequelae in children with Hib and pneumococcal meningitis.[117] A more recent, large-scale study from Malawi showed no benefit from dexamethasone as an adjuvant treatment.[118] In Latin America, a large trial examining the use of adjuvant glycerol and/or dexamethasone in childhood bacterial meningitis suggested that dexamethasone does not significantly affect mortality or the risk of neurological sequelae, but might prevent hearing loss in children with Hib meningitis.[119,120] A Cochrane meta-analysis from 2015 concluded that dexamethasone reduces

the overall risk of hearing loss in children, but does not significantly alter mortality or the risk of neurological sequalae.[115] In subgroup analyses, the beneficial effect of dexamethasone on hearing impairment was detected in children with Hib meningitis, but not in meningitis resulting from other bacteria.[115] Moreover, no beneficial effects were noted in low-income countries, whilst dexamethasone seemed to reduce the risk of both severe hearing loss and short-term neurological sequelae in HICs.[115]

In neonates, only two modern prospective trials have investigated the benefits of dexamethasone in bacterial meningitis, neither blinded and both featuring rather small sample sizes. The first study from Daoud *et al.* showed no benefit from adjuvant dexamethasone, whilst the latter conducted by Mathur *et al.* suggested a better survival amongst infants receiving dexamethasone.[121,122] However, a Cochrane meta-analysis considered these data of very low quality and no clear recommendations were stated in relation to the findings.[123]

In conclusion, dexamethasone is commonly recommended as an adjuvant treatment for older children in HICs, but not for neonates or in resource-limited settings.[18,87]

Glycerol

Glycerol (glycerine, 1,2,3-propanetriol) is an osmotic agent widely investigated as an adjuvant treatment for meningitis. Glycerol was first introduced in the 1960s in the treatment of elevated ICP in neurosurgical patients, and has since been used in the treatment of different conditions leading to cerebral oedema such as ischemic stroke.[124] Because cerebral oedema and an increased ICP frequently appear in patients with meningitis, the use of glycerol as an adjuvant has been rationalised. Although not entirely clear, the mechanism of action is usually explained by an increase of the serum osmolality and a subsequent flow of water across the blood–brain barrier from the brain parenchyma into the blood vessels, ultimately leading a decrease in ICP.[124] The increase in plasma osmolality possibly decreases CSF secretion, and other mechanisms might contribute.[124,125] In line with this theory, a small study of children with bacterial meningitis detected an increase in serum osmolality following glycerol administration.[126]

Whilst animal studies have not demonstrated a benefit from adjuvant glycerol treatment,[51,127] the results from clinical trials show somewhat conflicting findings. A Cochrane meta-analysis of five studies was updated in 2018, concluding that glycerol may reduce neurological deficiencies and deafness when used as an adjuvant treatment in meningitis, but does not seem to affect mortality.[128] This meta-analysis included one study amongst Malawian adults, and of the four trials in children, two were rather small. The remaining two studies were large, randomised, double-blind placebo-controlled trials with two-by-two factorial designs: the first, conducted in ten centres across Latin America, examined the use of dexamethasone and/or glycerol, whilst the second single-centre study from Malawi compared the use of adjuvant glycerol and/or paracetamol with a placebo.[119,129] Briefly, oral glycerol appeared to reduce the risk of severe neurological sequelae in Latin America, whilst neither a benefit nor harm was noted in Malawi. The trials differed, however, in terms of bacterial aetiology, HIV prevalence, the use of prehospital antibiotics and the occurrence of seizures before treatment, amongst others. Lastly, the only adult study included in

the above-mentioned meta-analysis has had a profound impact on the use of glycerol in bacterial meningitis and deserves separate mention: a double-blind randomised controlled trial examining the adjuvant use of glycerol in Malawian adults with suspected bacterial meningitis was stopped early because of an increased mortality in the glycerol-receiving study arm (86/136 [69%] and 61/125 [49%] succumbed by day 40 in the glycerol and placebo groups, respectively).[130]

In conclusion, glycerol is currently not recommended as an adjuvant treatment for bacterial meningitis, neither in children nor adults.

Paracetamol

Paracetamol is a widely used antipyretic agent, which seems to act by indirectly inhibiting the activity of several cyclo-oxygenase enzymes, both peripherally and in the CNS.[131] Traditionally, the anti-inflammatory effects of paracetamol have been considered inferior to those of other cyclo-oxygenase inhibitors. However, paracetamol also inhibits other peroxidase enzymes such as MPO, which contributes to the pathophysiology of several inflammatory conditions.[131] Consequently, paracetamol emerges as an interesting treatment candidate for inflammatory conditions such as sepsis or meningitis.

In critically ill adults, no conclusive benefit from the use paracetamol has been established, and some concerns have been raised due to its possible hypotensive effects.[132] In children with bacterial meningitis, several robust trials have examined the use of adjuvant paracetamol. The above-mentioned trial in Malawi, which applied a two-by-two factorial design of glycerol and paracetamol, failed to show any benefit from the adjuvant use of rectal paracetamol.[129] A previous trial from a tertiary hospital in Luanda, Angola, investigated the use of a 24-hour continuous cefotaxime infusion and/or oral paracetamol for two days as treatment for childhood bacterial meningitis.[83] Whilst no significant differences between the study treatments emerged in terms of the predefined endpoints, a *post hoc* analysis suggested an improved initial survival in paracetamol recipients, irrespective of the antibiotic dosing.[83]

Potential future adjuvant treatments

Potential future adjuvant options, evaluated primarily in experimental meningitis, include different agents affecting initial TLR signalling, cyto- and chemokines, antimicrobial proteins, complement function, the influx of leukocytes into the CNS, the actions of ROS and RNS and the resolution of inflammation, amongst others.[51,114] That said, the most promising likely options include the inhibition of MMPs and the use on nonbacteriolytic antibiotics. Previous studies and the accumulated evidence concerning MMP inhibitors are discussed in Chapter 2.3.3 below.

The administration of bacteriolytic antibiotics during bacterial meningitis induce the lysis of bacteria in the CNS, leading to a release of proinflammatory compounds such as LTA, peptidoglycan and pneumolysin from pneumococci and endotoxin or LPS from meningococci.[58] This further results in an inflammatory burst, the strength of which associates with unfavourable disease outcomes.[51] Whilst withdrawing antibiotic treatment ultimately leads to even poorer results, this pathophysiological knowledge gave rise to the concept of using nonbacteriolytic antibiotics as pretreatment of meningitis. Several experimental studies have

confirmed this hypothesis: pretreatment or treatment with nonbacteriolytic antibiotics seems to reduce the release of bacterial proinflammatory compounds and often also improves the prognosis.[58,114] Promising antibiotics include the older protein-synthesis inhibitors rifampicin and clindamycin as well as daptomycin.[114] Thus far, only one study has tested this hypothesis in a clinical setting. In a small, unblinded pilot trial in India, 40 children with bacterial meningitis were randomised to receive either conventional ceftriaxone treatment or a single dose of rifampicin 30 minutes prior to ceftriaxone and regular treatment thereafter.[133] No differences in disease outcomes were noted, but the CSF levels of TNF-α and two markers of neuronal damage (S100B and neuron-specific enolase) were lower in the intervention group. Although the idea of using nonbacteriolytic antibiotics as pretreatment carries several inconveniences, such as delaying the real antibiotic treatment and the possible promotion of antibiotic resistance, studies investigating this treatment modality appear warranted.

2.1.7.3 Supportive treatment

The appropriate fluid therapy during bacterial meningitis remains debated. Given that many children with meningitis present with hyponatraemia, the use of restricted fluid therapy has been recommended in order to handle the possibly increased secretion of antidiuretic hormone. However, randomised trials have not confirmed this hypothesis, and currently, normal maintenance fluids are commonly recommended.[87,104,106,134] The most recent trial, conducted in Papua New Guinea, failed to show a difference in outcomes between restricted oral fluid therapy and normal intravenous maintenance fluids in children with bacterial meningitis.[135] Yet, the study found evidence suggesting that both under- and overhydration associate with poor disease outcomes.

In general, the treatment of bacterial meningitis should attempt to optimise perfusion, oxygenation and homeostasis in the CNS. In practice, this is achieved by means of stabilising blood pressure and saturation levels, correcting electrolyte and glucose levels, treating convulsions and occasionally also monitoring and reducing ICP.[27,106]

2.1.8 Disease outcomes

2.1.8.1 Overall prognosis

Mortality due to bacterial meningitis in children widely differs between HICs and countries with resource-poor healthcare systems.[136] In Angola, the case-fatality rate (CFR) remains around 30% to 40% for non-neonates, and recent studies from Malawi have reported CFRs between 23% and 31%.[83,118,129,137]. A study amongst Guatemalan children under 5 years of age showed a CFR of 24%, whilst a multicentre study from Latin America suggested an overall CFR of 13% in children beyond the neonatal period.[119,138] In Europe and the United States the all-cause CFR for bacterial meningitis oscillates between 5% and 15%, although most recent studies have focused on aetiology-specific mortality.[4,139-142] Meningococcal meningitis typically carries a better prognosis than meningitis caused by Hib or *S. pneumoniae*.

Traditionally, neonatal meningitis has associated with a poorer prognosis compared to meningitis in older children.[15] Two more recently published studies from North America and the United Kingdom reported, however, overall CFRs of 7% and 8%, respectively, reflecting improvements disease

prognosis in HICs.[19,20] Progress in resource-limited settings, however, lags behind such gains.[143,144]

An unfortunate characteristic of bacterial meningitis is the high risk of neurological and audiological sequelae in survivors. Typical sequelae due to bacterial meningitis include sensorineural hearing loss, motor deficits, cognitive impairment, seizures, visual problems or other cranial nerve disturbances and hydrocephalus.[145] Globally, approximately one in five survivors is left with some kind of sequelae.[145]

Hearing loss is the most common sequela following meningitis, detected in up to one in three surviving children.[118,120,129,146,147] Motor deficits, cognitive impairment, seizure disorders, visual impairment and other findings such as hydrocephalus are commonly described as an entity, and are often divided into severe and milder forms of impairment.[145] In Angola and Malawi, the risk of any neurological sequelae in surviving children approaches 30% to 50%.[83,118] Morbidity in terms of postmeningitis sequelae seems, however, rather unequally distributed across the globe. For instance, a meta-analysis suggested that the risk of at least one severe sequela (including hearing impairment) was close to three times higher in the African region (25.1%) compared to the European region (9.4%).[145] Moreover, the same meta-analysis suggested that the risk of sequelae parallels the trend in mortality in terms of causative agents, being highest for pneumococcal meningitis.[145]

2.1.8.2 Risk factors for poor outcomes

A substantial amount of research has focused on identifying the risk factors for poor outcomes of childhood bacterial meningitis. These studies have identified a wide range of prognostic factors, related to the preceding clinical course and the patient age, the causative organism, as well as to the clinical presentation and the laboratory findings upon admission. Many of these variables appear related to each other, and the use of multivariate versus univariate analyses has consequently yielded varying results. By correctly identifying those children in greatest need of treatment and supportive care, the often scarce resources might be targeted adequately to optimise disease outcomes.

A systematic review published in 2010 aimed to summarise the numerous prognostic factors identified for childhood meningitis.[148] No pooled analyses were conducted due to the heterogeneity of data, but the authors attempted to summarise the findings by looking at the number of studies identifying a certain risk factor, allowing simultaneously for study quality. When excluding studies of low quality and those applying only univariate analyses, impaired consciousness upon admission alone was identified in more than one study as a predictive factor for death.[148] In addition, the occurrence of seizures, signs of cardiovascular shock, a low peripheral white blood cell count and a high CSF protein level predicted an increased risk of death in more than one study applying univariate analyses. Using a similar approach, a low CSF glucose and pneumococcal aetiology seemed to best associate with poor audiological outcomes, whilst impaired consciousness, the occurrence of seizures and pneumococcal aetiology were identified as risk factors for neurological sequelae. Whilst this review has its obvious limitations, it provides an intelligible overview of the possible risk factors for unfavourable outcomes.[148]

The largest prospective study of this review, looking at predictive factors of poor disease outcomes in a cohort of children with bacterial meningitis from Latin America, was published in 2008.[149] Whilst separately examining prognostic factors for death, death or severe neurological sequelae and death or any neurological sequelae, the child's presenting condition in terms of the Glasgow Coma Scale (GCS) score remained the only independent predictor of these three outcomes. Interestingly, the study suggested that a bacterial aetiology (*S. pneumoniae* vs. Hib) associated with mortality only in patients without a severely impaired consciousness (i.e., a GCS score >12); in these children, pneumococcal aetiology associated with a fivefold higher risk of death. However, if the child was more severely ill upon presentation, the aetiology did not affect the risk of death. Given this finding, the authors concluded that studies assessing the efficacy of treatment modalities should take into account the child's presenting condition in addition to the bacterial aetiology.[149] Another study from Angola identified impaired consciousness upon admission, severe dyspnoea upon admission and seizures during hospitalisation as independent risk factors for death.[137] Again, the causative agent seemed to be of secondary importance.

2.2 CONTINUOUS INFUSION OF BETALACTAM ANTIBIOTICS

Whilst antimicrobial resistance is emerging across the globe, rationalising antibiotic treatment has become a major subject of interest in recent decades. Antimicrobial stewardship has been implemented in many hospitals in order to control the evolution of resistant pathogens, and the search for new antimicrobial drugs continues. However, the use of existing antibiotics might also be optimised by changing ways of administration, taking into account the pharmacokinetic and -dynamic properties of a drug. The use of prolonged or continuous infusions of β-lactam antibiotics represents one such strategy, currently enjoying increasing use in the treatment of challenging infections.

2.2.1 Theoretical rationale

β-lactam antibiotics exert time-dependent killing of bacteria. Thus, the best parameter reflecting the therapeutic efficacy of β-lactams is the time during which the antibiotic concentration exceeds the minimum inhibitory concentration (MIC) of a given pathogen at the site of infection, often referred to as T>MIC.[150] In other words, T>MIC rather than the maximum concentration obtained most accurately predicts the efficacy of β-lactam antibiotic treatment (Figure 8).[151] Other antibiotics such as fluoroquinolones and aminoglycosides exert concentration-dependent killing: the higher the antibiotic concentration, the better the bactericidal effect.[150] In contrast, increasing the maximum antibiotic concentration will not result in much additional efficacy when using β-lactams, and, generally, concentrations of only three to five times the MIC are required for maximum bacterial-killing effects.[151,152]

The minimum T>MIC required for successful bacterial eradication depends on the characteristics of the chosen antibiotic, the causative agent and the host's immune status. Some β-lactams exhibit a post-antibiotic effect (PAE), meaning that the regrowth of

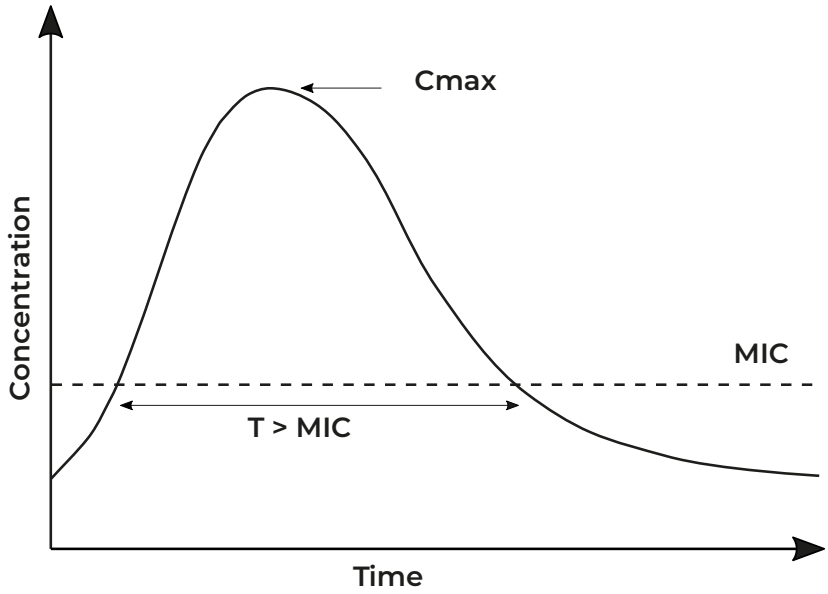

Figure 8 Pharmacodynamic variables related to antibiotic treatment. Abbreviations: Cmax, maximal antibiotic concentration; MIC, minimal inhibitory concentration; T > MIC, the time that the antibiotic concentration exceeds the MIC. Modified from MacVane *et al.*[153]

bacteria does not begin immediately when the antibiotic concentration falls below the MIC. The existence of a PAE depends on both the antibiotic and the pathogen: for instance, penicillins and cephalosporins demonstrate some PAE against staphylococci but usually not against Gram-negative bacteria. In the absence of neutrophils, the minimum T>MIC to achieve efficacy has been suggested to approach 90% to 100% for penicillins and cephalosporins when treating streptoccci.[151] Nonetheless, cefotaxime demonstrated a maximum killing effect on *Klebsiella pneumoniae* already when the antibiotic concentrations exceeded the MIC 60% to 70% of the time in neutropenic mice with a lung infection.[150] In nonneutropenic conditions, the minimum T>MIC is lower for successful bacterial eradication, since the host's immune response contributes to the overall effect. In studies of patients with otitis media, a T>MIC of at least 40% resulted in a bacteriological cure rate of roughly 85% to 100%.[150] However, some researchers have suggested that higher target T>MICs should be achieved in critically ill patients.[154] Generally, a larger T>MIC is required for cephalosporins than for penicillins, whilst carbapenems require the smallest T>MIC of β-lactams. This difference reflects the rate of bacterial killing, which is slowest for cephalosporins.[150]

Using the same total dose, the steady-state concentration obtained by a continuous infusion of β-lactams exceeds the minimum antibiotic concentration during bolus administration.[152] Thus, the use of a continuous infusion commonly results in higher

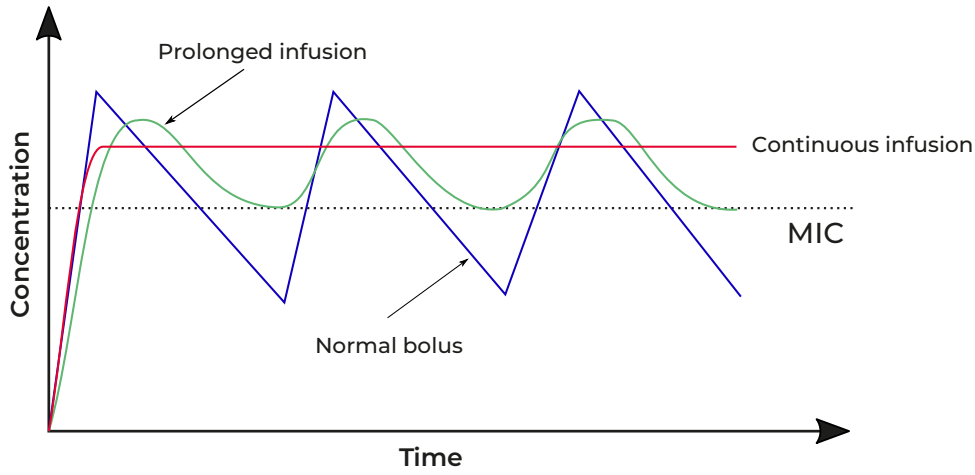

Figure 9 Serum antibiotic concentrations obtained using different dosing regimens. Abbreviations: MIC, minimal inhibitory concentration. Modified from Tamma *et al.*[155]

T>MICs (up to 100%) than when administering the antibiotic in boluses, provided that the steady-state concentration exceeds the MIC of the pathogen (Figure 9). Acknowledging that the bactericidal effect of β-lactams is largely independent of the maximum antibiotic concentration, these data favour the use of a continuous infusion in terms of pharmacokinetics.

With our current bolus dosing schedules for β-lactams, sufficient T>MICs are, however, obtained in most patients, which result in successful bacterial killing.[152] Yet, the use of continuous infusions has been recommended in specific situations in order to maximise T>MIC and ensure treatment efficacy.[156] First, a continuous infusion might provide an additional benefit when treating pathogens with reduced antibiotic susceptibility. When the MIC for a specific pathogen approaches the clinical susceptibility breakpoint, the advantage of a continuous infusion may become clearer as the required antibiotic concentrations increase.[156] Second, critically ill patients who present with an altered physiology might also represent one possible target population. In these patients, the distribution volume of a drug may increase due to the extravasation of fluids and aggressive fluid resuscitation. In addition, drug clearance may also increase, requiring a more accurate administration of antibiotics to achieve target concentrations.[157] Third, based primarily on experimental studies in neutropenic animals, some authors have argued that neutropenic patients might comprise a suitable population for the use of continuous infusions of antibiotics.[156]

As reviewed earlier, the release of bacterial compounds in the CSF during bacterial meningitis launches the host's immune response, the extent of which associates with the disease outcomes. Several experimental studies have shown that the release of proinflammatory bacterial compounds and the extent of the subsequent inflammatory burst increases when bacteriolytic antibiotics are administered: these antibiotics cause

the lysis of the bacteria, resulting in the rapid release of a large amount of bacterial compounds.[58] In general, high antibiotic concentrations seem to release fewer proinflammatory bacterial compounds than concentrations close to the MIC.[58,158,159] Thus, provided that a continuous infusion results in a stable antibiotic concentration remaining above the MIC for the entire treatment period, a continuous infusion might also be beneficial in reducing the release of proinflammatory compounds.

2.2.2 Previous clinical experience

The use of a continuous antibiotic infusion has been most extensively studied in critically ill adult patients in the ICU. Despite the theoretical advantages of a continuous infusion, clinical trials have failed to demonstrate a clear benefit from the use of this dosing regimen within the ICU. A Cochrane meta-analysis published in 2013 concluded that no significant differences in the measured outcomes were found between using continuous or intermittent infusions.[160] However, this meta-analysis comprised all kinds of infections requiring intravenous antibiotic treatment (also less severe diseases), and most of the studies included were evaluated to carry an unclear or high risk of bias.

Since 2013, several robust clinical trials examining the use of a continuous β-lactam infusion in the treatment of septic infections have been published. A smaller multicentre study (n = 60) suggested a benefit from the use of a continuous infusion regarding pharmacokinetic targets (T>MIC) and the clinical cure in adults with severe sepsis.[161] Encouraged by this result, a larger study (n = 432) was conducted across 25 different ICUs in Australia, New Zealand and Hong Kong, comprising a similar population of critically ill adults.[162] However, this study found no differences in survival or clinical cure between the use of a continuous infusion or conventional bolus dosing. The most recently published study, conducted in two ICUs in Malaysia, suggested that the use of a continuous infusion associated with better pharmacokinetic and clinical outcomes in adults with severe sepsis.[163] When compared to the above-mentioned larger trial, the Malaysian trial differed in terms of patient inclusion (not comprising patients on renal replacement therapy) and the causative agents, since the Malaysian study participants might have suffered from more pathogens commonly associated with reduced antibiotic susceptibility.[163] In conclusion, the use of a continuous infusion in the treatment of adult septic patients might be beneficial, but only in a properly chosen subpopulation.

Less data exist concerning the use of continuous β-lactam infusions in children. A review published in 2012 suggested that the currently available evidence does not suffice for determining the role of this dosing regimen of β-lactams in the paediatric population, since only one randomised trial satisfied the inclusion criteria.[164]

Most of the previously mentioned studies have comprised patients with septic infections, pneumonia and other infectious outside the CNS. The fact that the CSF constitutes a relatively immunocompromised space separated from systemic circulation by the blood–brain barrier crucially distinguishes bacterial meningitis from infections outside the CNS. The results from the above-mentioned trials should, therefore, not be extrapolated to the treatment of meningitis, but can be considered as background for the section that follows.

Not much data exist concerning the different dosing regimens of cefotaxime in the treatment of bacterial meningitis in children. In a small sample of 18 children receiving conventionally administered cefotaxime (200 mg/kg daily, divided into 4 doses), the traditional dosing regimen seemed to result in sufficient antibiotic concentrations in the CSF when treating meningitis caused by Hib, *N. meningitidis* and *S. pneumoniae*.[165] Whilst this is likely the case for most children, some concern has been raised concerning the pharmacokinetics of cefotaxime in critically ill children.[166] In adult neurosurgical patients with postoperative intracranial infections, the use of a continuous infusion associated with faster clinical improvement and better achievement of pharmacokinetic targets such as the CSF drug concentration above the MIC for the specific pathogen.[167] However, this study used cefepime instead of cefotaxime, and comprised a completely different range of bacteria than those usually causing community-acquired meningitis in children.[167]

Prior to writing this doctoral thesis, only one prospective study had investigated the use of a continuous β-lactam infusion in the treatment of bacterial meningitis. That study, carried out by our research group and applying a two-by-two factorial design, examined the use of a continuous intravenous infusion of cefotaxime and orally administered paracetamol in the treatment of childhood bacterial meningitis in Angola.[83] Comprising a total of 723 participants (median age, 14 months) with suspected bacterial meningitis, the children were randomised to receive either a continuous infusion of cefotaxime and oral paracetamol, a continuous infusion and oral placebo, conventional boluses of cefotaxime and oral paracetamol or conventional boluses and an oral placebo. The continuous infusion of cefotaxime lasted for 24 hours, whereafter conventional bolus administration continued in all study groups for at least 6 days. The daily dose of cefotaxime was 250 mg/kg in both dosing regimens. Oral paracetamol was given for 48 hours, the first dose being 30 mg/kg, followed by 20 mg/kg every 6 hours. In addition, all children received glycerol at a dose of 1.5 mL/kg (maximum dose, 25 mL), four times daily for two days. Briefly, children with signs and symptoms of bacterial meningitis and a cloudy CSF, CSF pleocytosis or a positive CSF Gram stain comprised the intention-to-treat population, whilst the per-protocol population was restricted to those with a confirmed diagnosis of bacterial meningitis.[83] Exclusion criteria consisted of more than one preceding dose of parenteral antibiotics, previous neurological disorders, a hearing impairment, trauma, intracranial shunts and immunosuppression except due to HIV. The primary outcomes were death, severe neurological sequelae or deafness at discharge from hospital.

Whilst no significant differences were detected regarding the predefined endpoints between the four treatment groups, a *post-hoc* analysis revealed that children who received both a continuous infusion and oral paracetamol exhibited higher survival rates for at least three days (Figure 10).

Moreover, in a predefined subgroup analysis of pneumococcal meningitis, a cefotaxime infusion with a placebo appeared to associate with a better prognosis in terms of the composite outcome of death or any neurological sequelae.[83] Because the continuous infusion was stopped after 24 hours and paracetamol administration after 48 hours, the transient improvement of survival in children receiving both interventions gave rise to doubts about the interventions possibly being too

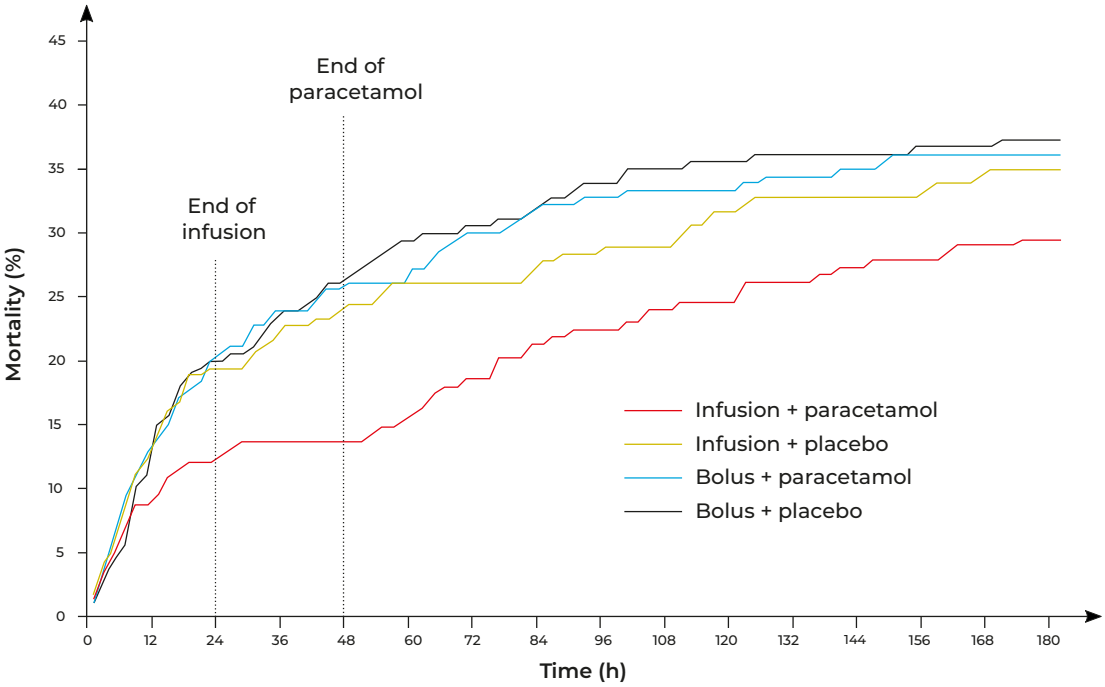

Figure 10 Mortality curves for the four different treatment groups in the per-protocol population of the study by Pelkonen et al.[83] Modified from Pelkonen *et al.*[83]

short in duration.[83,168] As a result, a follow-up trial applying a similar but longer treatment intervention was planned and recently conducted at the same institution, constituting one of the five studies in this doctoral thesis.

As previously noted in Chapter 2.1.7.2, earlier trials examining the use of paracetamol as an adjuvant treatment of childhood bacterial meningitis remain as scarce as studies assessing the potential benefit of a continuous β-lactam infusion. To provide the background for the clinical trial included in this thesis, Table 2 summarises the preceding relevant clinical studies on these two treatment modalities.

Table 2. Clinical studies examining the use of paracetamol or continuous β-lactam infusion as treatment for bacterial meningitis

Study	Design	Patients	Intervention	Outcome
Paracetamol				
Molyneux et al. (2014)[129]	Randomised, placebo-controlled, double-blind (2 x 2)	360 children at Queen Elizabeth Central Hospital (Blantyre, Malawi)	Rectal paracetamol for 42 hours vs. placebo, with or without oral glycerol	No benefit from the intervention
Pelkonen et al. (2011)[83]	Randomised, placebo-controlled, double-blind (2 x 2)	723 children at Luanda Children's Hospital (Luanda, Angola)	Oral paracetamol for 48 hours vs. placebo, with or without CI of β-lactam	No difference in the primary outcome. Post-hoc analysis suggested improved initial survival in paracetamol recipients
Continuous infusion				
Huang et al. (2014)[167]	Retrospective, comparative, nonrandomised	68 adults with postneurosurgical infections at Fujian Provincial Hospital (Fuzhou, China)	Intravenous cefepime every 12 hours vs. cefepime as CI	Faster clinical improvement and higher target antibiotic concentrations in patients receiving CI
Pelkonen et al. (2011)[83]	Randomised, placebo-controlled, double-blind (2 x 2)	723 children at Luanda Children's Hospital (Luanda, Angola)	Intravenous CI of cefotaxime for 24 hours vs. bolus administration with or without oral paracetamol	No difference in the primary outcome. Post-hoc analysis suggested improved initial survival in children receiving both CI and paracetamol, and better composite outcomes in children with pneumococcal meningitis receiving only CI vs. bolus

Abbreviations: CI, continuous infusion

2.3 MATRIX METALLOPROTEINASES (MMPS)

2.3.1 Overview

Matrix metalloproteinases constitute a family of potent proteolytic enzymes widely expressed in many different cell types throughout the human body. This group of enzymes is structurally similar, but functionally and genetically rather distinct. Thus far, 23 different MMPs have been identified in humans. Based on their structure and function, MMPs can be further divided into nine subgroups, including the collagenases (MMP-1, MMP-8, MMP-13) and gelatinases (MMP-2, MMP-9), amongst others.[169-171]

MMPs were originally recognised as enzymes that degrade components of the extracellular matrix. However, most known MMP substrates today are of nonmatrix origin, whereby the current understanding indicates that MMPs predominately function to alter the release and activity of different cytokines, chemokines and growth factors.[169,170,172] Studies amongst MMP-knockout mice have revealed surprisingly mild phenotypes, often

showing abnormalities only during pathological challenges; thus, in general, the loss of a single MMP enzyme does not appear to endanger normal development.[169] Conversely, MMPs seem to play an important role in many different pathological conditions such as wound healing, inflammatory and infectious diseases, cancer development and vascular diseases.[169-171] The expression of many MMPs remains low under stable conditions, but is strongly upregulated in response to inflammation or tissue injury.

2.3.2 Structure and regulation

Most MMPs share a similar molecular structure, including an N-terminal signal peptide, a pro-peptide domain regulating the activity of the enzyme, and a catalytic domain with a zinc atom. Furthermore, all except three MMPs have a hemopexin-like C-terminal domain connected to the catalytic centre by a flexible hinge region (Figure 11).[173]

A conserved cysteine residue in the pro-peptide domain interacts covalently with the catalytic zinc atom rendering the enzyme inactive; a disruption of this bond is required for catalytic activity. The hemopexin-like domain serves as a binding site for tissue inhibitors of metalloproteinases (TIMPs), whilst the signal peptide is required for the secretion of the enzyme.[170,173] In addition to these conserved regions, MMPs contain other functional regions depending on their characteristics and substrate specificity: for instance, membrane-type MMPs have specific regions for anchoring the enzyme to the cell membrane.[170]

Because MMPs are capable of potentially harmful actions, their activity is tightly regulated at several stages, from gene expression to activation of the enzyme (Figure 12). First, MMP gene expression remains typically stable and low-level under normal healthy conditions. Following appropriate stimuli, proinflammatory cytokines such as TNF-α and IL-1 (amongst others) enhance the transcription of MMPs.[173] MMPs differ, however, in terms of promoter regions and the characteristics of expression: the production of certain MMPs is strongly inductive (MMP-8, MMP-9), whilst others (MMP-2) are more constitutively expressed.[169] Recent studies have suggested that epigenetic and post-transcriptional modification also contribute to the regulation of MMP gene expression.

Compartmentalisation comprises the second step in MMP regulation. In other words, the localisation of the MMP restricts the activity of the enzyme. For instance, MMP-8 is secreted from specific intracellular granules in response to the appropriate stimuli, eventually enabling the activation of the enzyme and its further proteolytic actions.[173,174] Compartmentalisation also refers to the membrane-bound nature of

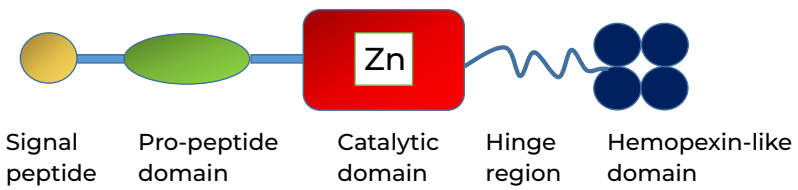

Figure 11 Structure of matrix metalloproteinases (MMPs).[169]

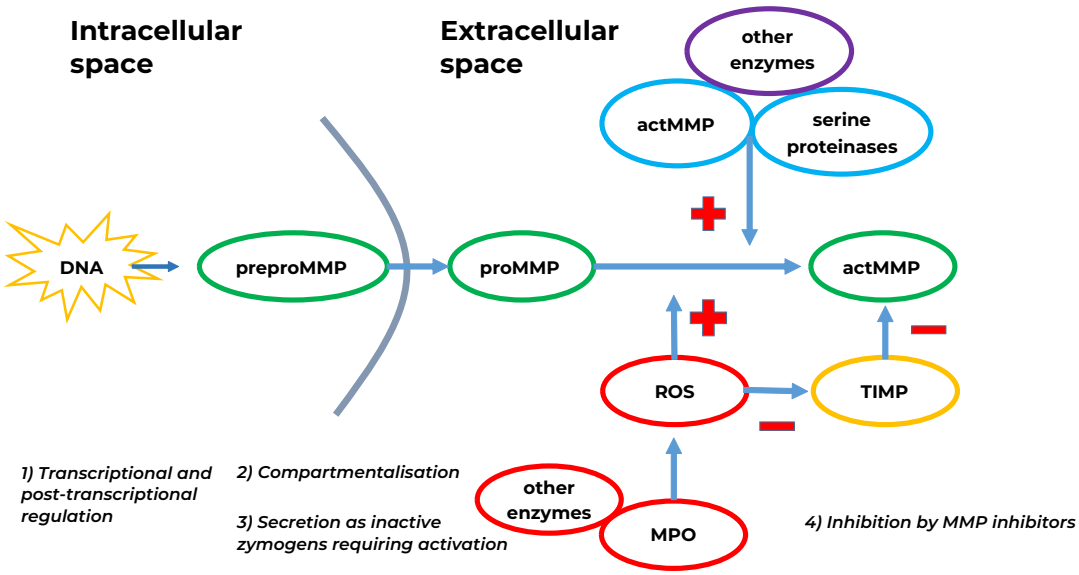

Figure 12 Regulation of matrix metalloproteinase (MMP) activity.[169,170] Abbreviations: actMMP, activated MMP; MPO, myeloperoxidase; preproMMP, pre-form of proMMP; proMMP, pro-form of MMP.

many MMPs, which focuses the enzymes' activities to the pericellular space.[169]

Third, most MMPs are secreted as inactive zymogens, requiring separate activation before becoming catalytically operational. As mentioned above, a disruption of the bond between the pro-peptide domain's cysteine residue and the catalytic centre is required. This can be accomplished in different ways: the pro-peptide domain can be proteolytically removed by enzymes such as activated MMPs or other proteases; or the interaction between the cysteine residue and the catalytic centre might be altered through chemical or allosteric modification by ROS or other agents.[64,169]

Fourth, the proteolytic actions of now activated MMPs can finally be inhibited by either broad-spectrum protease inhibitors or specific MMP inhibitors. Four specific MMP inhibitors exist, known as TIMP-1, -2, -3 and -4. These TIMPs show variable tissue expressions and slight differences in the substrate specificity, although all four can inhibit all MMPs at a molar ratio of 1:1.[169,173] Indeed, the MMP:TIMP ratio for a specific enzyme has been suggested as an accurate measure of the enzyme's proteolytic potential.[175] In addition to MMP inhibition, TIMPs also appear to exert other actions such as MMP transportation and focalisation.

2.3.3 Matrix metalloproteinases (MMPs) in bacterial meningitis

The role of MMPs in the pathogenesis of bacterial meningitis began to emerge in the 1990s, when both animal and human studies suggested an increase in MMP-9 (gelatinase B) in the CSF during bacterial infection.[176-178] Since then, much research has been conducted on the effects of MMPs during bacterial meningitis, primarily focusing on MMP-9 and the collagenase MMP-8.

Both MMP-8 and MMP-9 seem to take part in the breakdown of the blood–brain barrier. In human brain microvascular endothelial cell cultures, MMP-8 contributed to the cleavage of occludin and cell detachment during meningococcal infection, possibly reflecting a disruption to the blood–brain barrier.[179] A similar effect has been suggested for MMP-9, although the evidence remains less conclusive.[178,180,181] In addition to MMP-8 and MMP-9, MMP-2 also appears capable of disrupting the blood–brain barrier.[182] However, due to the primarily constitutive expression profile of MMP-2, its clinical significance in meningitis remains unclear. In general, it seems likely that MMPs contribute to the breakdown of the blood–brain barrier, an effect traditionally attributed to MMPs in the context of meningitis.

The effects of MMPs during bacterial meningitis are probably more diverse, recognising their ability to alter the host's inflammatory response.[170] MMP activity in the CSF during bacterial meningitis has been repeatedly, although not consistently, associated with the CSF white cell count.[175,183-185] Often, this is assumed to reflect the cellular origin of the enzyme. However, because MMPs are capable of altering the function of chemokines and cytokines, the above-mentioned correlation may also reflect the proinflammatory effect of MMPs, further promoting leukocyte influx into the CSF. Indeed, alongside polymorphonuclear leukocytes, parenchymal brain cells appear to contribute to the expression of MMPs in the CSF during meningitis.[183]

This background has prompted several experimental murine studies examining the use of MMP inhibitors as an adjuvant treatment of meningitis. The results from these studies suggest a benefit from MMP inhibition in experimental meningitis, reflected as improved survival, attenuated neuronal cell death, restored function of the blood–brain barrier and an improved learning function in the animals studied.[186-190] Recently, different MMP inhibitors have been combined with the nonbacteriolytic antibiotic daptomycin as an adjuvant treatment of murine experimental meningitis, again showing promising results.[191,192] Such studies have, however, used different MMP inhibitors with a mostly broad-spectrum substrate specificity. The optimal spectrum of MMP inhibition, thus, remains unknown. Moreover, none of these treatments studied have been evaluated in a clinical setting, leaving many questions unanswered and their efficacy open to debate.

Several clinical studies have, however, examined the relationship between CSF MMPs and disease outcomes in patients with bacterial meningitis. A small series of 27 children with bacterial meningitis indicated an association between high levels of CSF MMP-9 and post-meningitis neurological sequelae, whilst no such relationship was detected for CSF MMP-8.[185] A larger study amongst 264 children with bacterial meningitis suggested a similar association of MMP-9 to poor outcomes: the median CSF MMP-9 concentration upon admission was 2.7 times higher in children who died compared to survivors.[175] The authors of the latter study also reported that elevated levels of CSF TIMP-1 associated with neurological sequelae in survivors, and that the relationship of MMP-9 to TIMP-1 was inferior to MMP-9 alone in predicting death.

In conclusion, current evidence indicates that MMPs play a role in the pathogenesis of bacterial meningitis. Clinically, this knowledge has not yet contributed to better treatment options or improved diagnostics, although promising results have been obtained in experimental settings.

2.4 CATHELICIDIN

2.4.1 Overview

Cathelicidins comprise a group of antimicrobial proteins, comparable to other antimicrobial proteins such as human defensins and the lactoferrin-derived peptide lactoferricin. Cathelicidins have been found in most mammal species studied thus far, sharing a conserved N-terminal of the protein. Whilst most species express several cathelicidin proteins, the only human cathelicidin, hCAP18, was discovered in 1995. Similar to the cathelicidin proteins of other species, a 37-amino acid cationic peptide located in the C-terminal end harbours the antimicrobial activity of hCAP18. This peptide, also called LL-37, requires proteolysis from hCAP18 to become activated. Hereafter in this thesis, the term cathelicidin will refer to the human cathelicidin LL-37 unless otherwise stated.[193]

Cathelicidin is expressed by cells in contact with the environment, mainly different kinds of epithelial cells and immune system cells. Together with ROS, defensins and other antibacterial agents, it contributes to the first-line defence against bacteria. Immune cells such as neutrophils and natural killer cells produce the precursor of cathelicidin and store it in specific granules, which are then secreted in response to an infection or tissue damage.[193,194] The expression of cathelicidin may, depending upon the tissue and cell type, either be constitutive or inducible.[193] In the 2000s, the expression of cathelicidin was shown to be regulated by vitamin D, mediated by the vitamin D receptor (VDR) binding to the vitamin D response element in the promoter region of the cathelicidin gene.[194-196] To bind to the VDR and to promote the expression of cathelicidin, circulating 25-hydroxyvitamin D (25-OHD) needs to be converted into biologically active 1,25-dihydroxyvitamin D. Thus, the TLR-mediated stimuli of monocytes and macrophages leads, alongside the expression of VDR, to the upregulation of the enzyme 1α-hydroxylase (CYP27B1), which converts inactive 25-OHD into its active form.[195] Providing a direct link between vitamin D metabolism and our immune defence, this discovery has received much attention. Overall, however, the expression of cathelicidin is subject to rather complex regulation, affected by different cytokines, glucocorticoids and bacterial compounds.[62,193] Furthermore, because cathelicidin is secreted as part of hCAP18, its final activation requires cleavage from this precursor protein by a protease.

Once secreted and activated, cathelicidin appears to exert a variety of different functions, often called a pleiotropic or multifunctional peptide. Originally, cathelicidin was shown to be capable of binding and inactivating LPS, and exerting direct antibacterial effects. The antibacterial effect was later attributed to the pore-forming capacity of cathelicidin, thereby disrupting the bacterial membrane homeostasis; this effect targets a wide variety of both Gram-negative and Gram-positive bacteria.[193] Moreover, cathelicidin seems to prevent the formation of biofilm by *Pseudomonas aeruginosa*, as well as exert both antifungal and antiviral effects.[193] Besides these direct anti-infective effects, cathelicidin possesses a multidimensional immunomodulatory role. Acting through several receptors, it modifies our immune responses in both pro- and anti-inflammatory directions, affects the differentiation of T cells and acts as a chemotactic agent. Finally, cathelicidin also appears to play a role in wound healing, angiogenesis and cancer pathogenesis.[193]

Altogether, the effects of cathelicidin fall into two groups. The antimicrobial and LPS-binding properties represent the direct anti-infective effects as part of our first-line immune defence, whilst the immunomodulatory effects comprise a separate entity. Generally, the actions and amount of cathelicidin should presumably remain balanced and well-controlled, since both under- and overexpression of cathelicidin associates with different diseases.[193]

2.4.2 Cathelicidin in bacterial meningitis

Given that cathelicidin takes part in the first-line defence against invading bacteria, it seems likely that this peptide contributes to the host defence in bacterial meningitis. Indeed, an increased concentration of cathelicidin has been documented in the CSF of patients with both bacterial and tuberculous meningitis.[62,197] Based on these reports, the magnitude of CSF cathelicidin expression seems lower in tuberculous than bacterial meningitis, with mean values of roughly 5 ng/mL versus 80 ng/mL, respectively. More comprehensive data on the role of this peptide during meningitis have been obtained from experimental studies. In addition to circulating immune cells, at least astrocytes, microglia and meningeal epithelial cells appear to contribute to the production of cathelicidin during meningitis.[62,198] All of these cell types seemingly express cathelicidin rather swiftly when stimulated either by proinflammatory cytokines such as TNF-α and IL-1β or by bacterial components. Mice deficient in the murine analogue of cathelicidin (cathelin-related antimicrobial peptide or CRAMP) exhibited a higher mortality, higher bacterial titers and higher levels of proinflammatory cytokines during bacterial meningitis compared to wild-type mice.[199] Furthermore, treatment with an intrathecal infusion of CRAMP resulted in an increased survival and attenuated inflammatory responses in mice with bacterial meningitis.[200] Whilst no differences were noted in the granulocyte infiltration of the meninges between the treatment groups, the authors argued that the effects of cathelicidin appeared unmediated via neutrophil recruitment, but through more direct antibacterial or immunomodulatory effects. The attenuated inflammatory response in CRAMP-treated mice suggests that cathelicidin might exert beneficial anti-inflammatory effects during the immune response to bacterial meningitis.[200]

2.4.3 Vitamin D and immunity

Providing the first example of the immunoregulatory role of vitamin D, *Mycobacterium tuberculosis* infections were successfully treated with sun therapy and cod liver oil supplements in the preantibiotic era.[201,202] As previously mentioned, the subsequent discovery that vitamin D regulates the expression of cathelicidin established a direct link between vitamin D metabolism and our immune defence. This elegant cascade, where a TLR-mediated stimuli leads to the enhanced local production of 1,25-dihydroxyvitamin D, the upregulation of VDR and, finally, to the increased production of cathelicidin, is nonetheless only one of the immunoregulatory roles of vitamin D (Figure 13).

An immune cell needs to express VDR to be directly affected by vitamin D. The conversion of 25-OHD into biologically active 1,25-dihydroxyvitamin D represents another requisite step; if the cell does not express 1α-hydroxylase, the activation of vitamin D relies on other nearby cells (paracrine activation) or on the classical activation

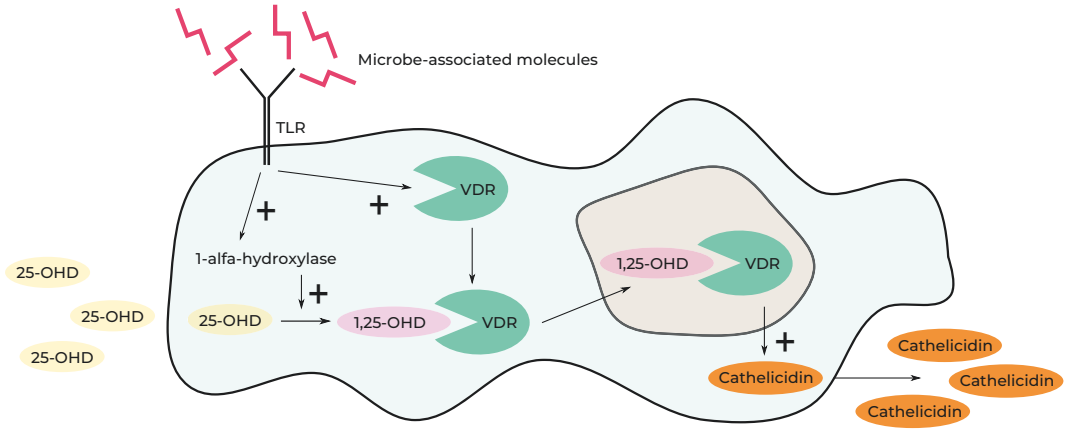

Figure 13 The vitamin D–mediated upregulation of cathelicidin expression in macrophages upon microbial stimuli. Abbreviations: 1,25-OHD, 1,25-dihydroxyvitamin D; 25-OHD, 25-hydroxyvitamin D; TLR, Toll-like receptor; VDR, vitamin D receptor. Modified from Zasloff.[203]

pathway in the kidney (endocrine activation). Interestingly, neutrophils represent one such group of cells that appear to express VDR, but not 1α-hydroxylase.[204]

Alongside promoting the production of cathelicidin, vitamin D appears to shift our immune responses from proinflammatory towards more tolerant. By affecting both antigen-presenting cells and T cells themselves, vitamin D promotes the formation of regulatory T cells, which suppress inflammatory responses launched by other immune cells.[204,205] Vitamin D promotes the production of anti-inflammatory cytokines and suppresses the formation of proinflammatory cytokines in different T cells, and inhibits B cell maturation. Given these effects, vitamin D appears to play a role in the development of different autoimmune diseases.[204,205]

Acknowledging the various immunomodulatory effects of vitamin D, it is unsurprising that vitamin D deficiency has been linked to poor outcomes for many infectious diseases. Best documented is the association between vitamin D deficiency and an increased risk of sepsis and overall mortality in critically ill adults.[206,207] In terms of mortality, similar results have been reported for children.[208] Although somewhat more controversial, low levels of vitamin D have also been associated with an increased rate of respiratory tract infections.[209] In the context of meningitis, vitamin D deficiency seems to be associated with tuberculous, but not cryptococcal infection.[210,211] In terms of bacterial CNS infections, an experimental study suggested an increased mortality in vitamin D deficient mice with meningoencephalitis, but no clinical data exist on the impact of vitamin D status on the risk or outcomes of bacterial meningitis.[212]

Although vitamin D evidently regulates the expression of cathelicidin, their relationship remains controversial in a clinical setting. Some studies have suggested a weak correlation between vitamin D and cathelicidin in plasma, whilst others reported no relationship between them.[213-216] Although differences in study populations and methodology may partly account for these discrepancies, such findings might also indicate that vitamin D remains merely one of several factors regulating cathelicidin synthesis.

3 OBJECTIVES

In general, this doctoral thesis aimed to investigate the potential benefit of a continuous antibiotic infusion combined with paracetamol in the treatment of childhood bacterial meningitis, to study the expression and potential prognostic value of different inflammatory markers in the CSF of children with bacterial meningitis and to evaluate the impact of children's vitamin D status on the outcomes of this disease.

The specific objectives were as follows:

I) To evaluate whether a continuous four-day infusion of cefotaxime combined with oral paracetamol compared to conventional treatment improved the prognosis of bacterial meningitis in children,

II) To clarify how CSF MPO, MMP-8, MMP-9 and TIMP-1 relate to each other during childhood bacterial meningitis and to investigate whether these inflammatory mediators associate with the outcomes of disease.

III) To investigate the expression of cathelicidin, its dynamics and potential prognostic value in the CSF during bacterial meningitis in children.

IV) To examine the relationship between the CSF bacterial burden and the cathelicidin expression in children with bacterial meningitis.

V) To evaluate whether serum vitamin D levels associate with the outcomes of bacterial meningitis in children and to assess the potential relationship between serum vitamin D and CSF cathelicidin.

4 SUBJECTS AND METHODS

4.1 STUDY OF MODIFIED MENINGITIS TREATMENT (STUDY I)

4.1.1 Design and implementation

This randomised, placebo-controlled, double-blind study employed a parallel-group design. It was conducted at the Hospital Pediátrico David Bernardino in Luanda, Angola over a 5-year period (22 January 2012–21 January 2017). Children aged between 2 months and 15 years with symptoms and signs suggestive of bacterial meningitis on whom a lumbar puncture was performed were assessed for eligibility. They were included in the study if the CSF appeared cloudy, was positive by Gram staining or showed at least 50 leukocytes per mm^3, these criteria being similar to our previous study in Angola.[83] Pretreatment with more than one dose of a parenteral antibiotic, previous known neurological abnormalities or hearing impairment, immunosuppression, active tuberculosis or known hepatic disease resulted in exclusion. Bacterial meningitis was considered confirmed if bacteria were detected in the CSF by bacterial culture or PCR; a child had symptoms and signs compatible with bacterial meningitis and a positive blood culture; or a child showed compatible symptoms and at least two of the following criteria: CSF pleocytosis ≥100 leukocytes per mm^3 (predominantly polymorphs), a positive CSF Gram stain result, a positive CSF latex agglutination test or serum C-reactive protein level ≥40 mg/L.

The sample size was estimated based on our previous trial.[83] Assuming a 13% decrease (from 27% to 14%) in mortality using the study treatment amongst children with confirmed bacterial meningitis, at least 165 patients were required in each treatment arm. Due to several possible confounding factors, we intended to enrol a total of 400 patients. Nonetheless, the study protocol stated that the enrolment of patients would stop when 5 years had passed. The ethics committee of the hospital in Luanda approved the study protocol on 19 January 2012. Before enrolment, the attending physician explained the study to the child's legal guardian, and informed consent was obtained from all participants.

Randomisation was completed for 500 patients by means of a computer-generated list of random numbers in fixed blocks of 20, allocating patients to one of the two treatment groups in a 1:1 ratio without stratification. Numbered envelopes with instructions for the patient's specific treatments were stored in a box in the hospital ward, and these treatments were individually prepared by a study nurse not otherwise involved in patient care. Thus, the investigators, the treating physicians and the patient remained blinded to the treatment. All children received both infusions and boluses in order to mask the treatment intervention.

4.1.2 Interventions and additional treatment

This study examined the use of a four-day continuous infusion of cefotaxime combined with oral paracetamol as a potential treatment for bacterial meningitis compared to conventional boluses of cefotaxime, four times daily, combined with an oral placebo. The total dose of cefotaxime, 250 mg/kg per day, was consistent across treatment groups, and regular saline served as a placebo preparation for cefotaxime. The amount of intravenous placebo (either as boluses or continuous infusions) was similar to that of the corresponding dose of cefotaxime.

The first dose of paracetamol was 30 mg/kg, followed by 20 mg/kg every 6 hours for 4 days. Effervescent tablets of paracetamol (Panadol®) dissolved in water were used, and regular drinking water served as its placebo. The oral placebo was administered in the same quantity as the corresponding dose of paracetamol.

4.1.3 Outcome measures

The primary outcome of this study was mortality by day 7 from treatment initiation. Later in-hospital mortality was included as a secondary outcome, recorded both in terms of the exact time of death since treatment initiation as well as the total number of deaths in the two treatment groups at discharge from hospital. The composite outcomes of death or severe neurological sequelae (blindness, hydrocephalus requiring a shunt, quadriparesis or severe psychomotor retardation) and death or any neurological sequelae (hemi- or monoparesis, psychomotor retardation of any degree or ataxia, in addition to the above-mentioned sequelae) were recorded on day 7 and at discharge from hospital, and comprised secondary outcomes.

4.1.4 Statistical methods

Differences concerning the primary and secondary outcomes between the treatment groups were analysed using a chi-square test or using the Fisher's exact test in the case of low expected cell values in the 2 x 2 table. Survival and the time of death of participants in the two treatment groups were assessed through Kaplan-Meier analysis, and the log-rank test was used to detect potential differences. The impact of the intervention on the primary and secondary outcomes was presented as risk ratios with 95% confidence intervals (CIs), and the absolute risk differences with 95% CIs were obtained using a two-sample z-test.

The impact of the intervention on the measured outcomes was calculated separately for the per-protocol and intention-to-treat populations. The intention-to-treat population comprised all enrolled children who were started on treatment medications, whilst the per-protocol population included children with confirmed bacterial meningitis who received the complete allocated treatment and did not present with any exclusion criteria.

In the subgroup analyses, interaction tests were conducted using logistic regression analyses. All analyses were predefined in the study protocol. We consistently used a two-sided hypothesis in order to detect potential harms or adverse effects from the intervention. The level of statistical significance was set at $p < 0.05$, and the statistical analyses were performed using IBM's SPSS Statistics software, version 24 (IBM Corp., NY, US).

4.2 STUDIES OF POTENTIAL PREDICTIVE FACTORS FOR MENINGITIS OUTCOMES (STUDIES II–V)

4.2.1 Patient data

The patient data for retrospective studies II through V originate from a large multicentre clinical trial conducted in Latin America between 1996 and 2003.[119] This prospective, randomised, double-blind study investigated the use of dexamethasone, glycerol or their combination as adjuvant treatments for childhood bacterial meningitis. The participating centres comprised 10 institutions in Argentina, Brazil, the Dominican Republic, Ecuador, Paraguay and Venezuela,. The study protocol was approved by all local ethics committees, and informed oral consent was obtained for all participants.

Patients aged between 2 months and 16 years with suspected bacterial meningitis were assessed for eligibility. Bacterial meningitis was considered confirmed if any of the following criteria were fulfilled: a positive CSF bacterial culture, a positive blood bacterial culture with CSF findings typical for bacterial meningitis, or a positive CSF latex agglutination test and characteristic CSF findings. Bacterial meningitis was also diagnosed in patients with symptoms and signs compatible with bacterial meningitis fulfilling at least three of the following criteria: a CSF white blood cell count ≥1000 cells/mm3, a CSF glucose level <40 mg/dL (<2.2 mmol/L), a CSF protein level ≥40 mg/dL, a serum C-reactive protein level ≥40 mg/L or a blood white cell count >15 000 cells/mm3. The exclusion criteria included a recent head injury or neurosurgical procedure, a previous neurological disease, a known hearing impairment, immunosuppression or more than one received dose of parenteral antibiotic before inclusion. In total, 654 patients were included in the study (Figure 14).

All patients received 80 to 100 mg/kg ceftriaxone daily for 7 to 10 days as the antibiotic treatment, but were randomised re-

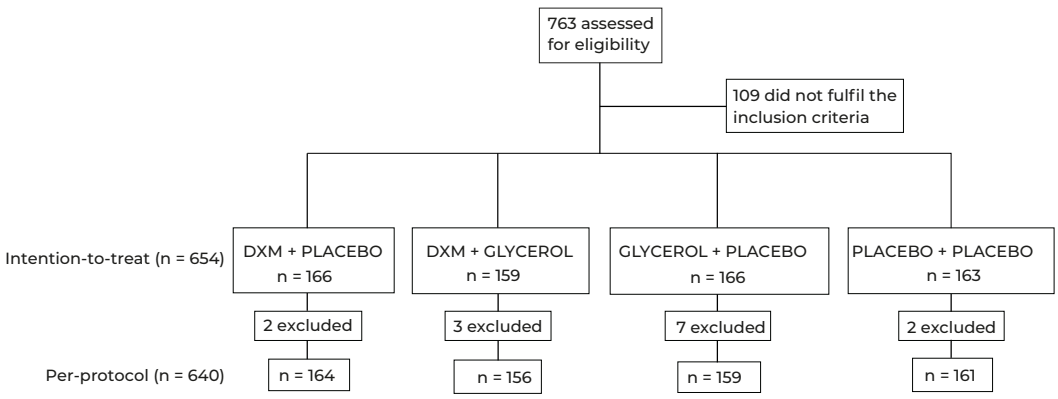

Figure 14 Flowchart of the study population in Latin America. Abbreviations: DXM, dexamethasone. Modified from Peltola *et al.*[119]

garding the adjuvant treatment to one of four treatment groups: oral glycerol and intravenous placebo, oral glycerol and intravenous dexamethasone, oral placebo and intravenous dexamethasone or oral and intravenous placebo. More detailed information on the randomisation and on the dosages administered are available in the original article.[119]

The disease outcomes were carefully registered. At discharge, a thorough neurological examination was completed on all survivors. Severe neurological sequelae were defined as hydrocephalus requiring a shunt, blindness, quadriparesis or quadriplegia or severe psychomotor retardation. In addition, any neurological sequelae comprised also milder deficits such as ataxia, hemiparesis, monoparesis or moderate psychomotor retardation. Retrospectively, the outcomes were further graded using the modified Glasgow Outcome Scale (GOS), with scores ranging from 1 to 5, where a higher score reflects a better outcome.[217]

Upon discharge or shortly thereafter, the ability to hear was tested using brainstem-evoked response audiometry or traditional pure-tone audiometry.[218] When local resources permitted, at least three hearing thresholds (40 dB, 60 dB and 80 dB) were evaluated. The results obtained with brainstem-evoked response audiometry were interpreted by an external audiologist, blinded to all other patient details. For the studies summarised here, the audiological outcome was described as the combined hearing threshold in both ears, as well as categorised into one of five ordinal categories using the hearing threshold of the better ear (1 = severest impairment, >80 dB; 2 = severe impairment, 80 dB; 3 = moderate impairment, 60-79 dB; 4 = mild impairment, 41-59 dB; and 5 = no impairment, ≤40 dB).

Serum samples were obtained upon admission and later if clinically warranted. CSF samples were obtained both upon admission and 12 to 24 hours after treatment initiation. After primary analyses, the samples were frozen to a minimum of -20 C° and transported to Finland for later use. In studies III and IV, both CSF samples were analysed in order to detect potential differences in CSF cathelicidin expression in response to treatment, whilst study II included only the diagnostic CSF samples obtained upon admission.

Studies II and III comprised subgroups of patients fulfilling the criteria for bacterial meningitis regardless of etiological confirmation, for whom the CSF was available for further analyses. Study III comprised only patients treated at Clínica Infantil Dr. Robert Reid Cabral in Santo Domingo in the Dominican Republic, whilst patients from five different centres were included in study II.

Study IV comprised a group of patients for whom the CSF bacterial genome count was previously analysed.[219] These patients consisted of children with confirmed Hib, meningococcal or pneumococcal meningitis. Finally, study V included patients with aetiologically confirmed bacterial meningitis, for whom a frozen serum sample (obtained upon admission) was available for the serum 25-OHD determination.

4.2.2 Laboratory methods

4.2.2.1 Gelatin zymography (study II)

The levels of MMP-9 activity in the CSF were detected using gelatin zymography, performed at the Scientific Laboratory of Oral and Maxillofacial Diseases (University of Helsinki, Helsinki University Hospital,

Helsinki, Finland). This technique, based on a modification of the method described by Lindberg *et al.*,[183] makes use of the gelatinolytic characteristics of the enzyme to assess its level in a specific sample. Briefly, 11% sodium dodecyl sulphate–polyacrylamide gels were impregnated with gelatin (1 mg/mL) as the substrate. After preincubation with a nonreducing Laemmli's sample buffer, electrophoresis was performed. The gels were then washed for 30 min twice with 50mM Tris-HCl buffer solutions, pH 7.5, containing 2.5% Tween 80 and 0.02% NaN3; the second wash was also supplemented with 0.5mM CaCl2 and 1µM ZnCl2. Finally, the gels were incubated overnight at 37°C in 50 mM Tris-HCl buffer, pH 7.5, containing 0.02% NaN3, 0.5mM CaCl2 and 1µM ZnCl2.

Thereafter the gels were stained with 1% Coomassie Brilliant Blue R-250. The subsequent gelatinolysis resulted in the formation of clear bands on the blue-stained gels, which were then evaluated with a Bio-Rad Model GS-700 Imaging Densitometer using the Bio-Rad Quantity One program (Bio-Rad laboratories, Hercules, CA, USA). Specific antibodies for MMP-9 (Calbiochem, Merck KGaA, Darmstadt, Germany) were used to confirm the molecular form of the enzyme employing the Western blot method.

4.2.2.2 Enzyme-linked immunosorbent and immunofluorometric assays (studies II–V)

The concentrations of MPO, TIMP-1 and cathelicidin in CSF were assessed using commercially available enzyme-linked immunosorbent assay (ELISA) kits. The following kits were used: MPO ELISA (Immunodiagnostik AG, Bensheim, Germany) for MPO, Amersham TIMP-1 Human Biotrak ELISA systems (Amersham Biosciences, GE Healthcare, Buckinghamshire, UK) for TIMP-1 and the Human LL-37 ELISA kit (Hycult Biotech, Uden, the Netherlands) for cathelicidin.

For the MPO kit, the interassay coefficient of variation was <3 % and the detection limit 0.294 ng/mL. The corresponding values for the TIMP-1 kit were <12 % and 1.25 ng/mL, respectively. These values were obtained in previous in-house comparisons. For the Human LL-37 ELISA kit, the manufacturer reports an interassay coefficient of variation of <15 % and a detection limit of 0.1 ng/mL.

The concentration of MMP-8 in the CSF was measured using a time-resolved immunofluorometric assay (Medix Biochemica, Espoo, Finland). Whilst using the monoclonal MMP-8-specific antibodies 8708 and 8706 as the catcher and tracer antibody, respectively, the latter was labelled with europium-chelate. After dilution and incubation, an enhancement solution was added and the fluorescence was measured after 5 min using a 1234 Delfia Fluorometer (Wallac, Turku, Finland). The assay showed an interassay coefficient of variation of 7.3% and a detection limit of 0.08 ng/mL.

The analyses of MMP-8, MPO and TIMP were conducted at the Scientific Laboratory of Oral and Maxillofacial Diseases (University of Helsinki, Helsinki University Hospital, Helsinki, Finland), whilst the cathelicidin concentrations were assessed in Sture Andersson's laboratory (Children's Hospital, Pediatric Research Center, University of Helsinki, Helsinki University Hospital, Helsinki, Finland).

4.2.2.3 Real-time PCR (study IV)

The real-time PCR methodology for quantifying the CSF bacterial genome count in pa-

tients with Hib or pneumococcal meningitis was published previously.[219] For the real-time PCR analysis of *N. meningitidis*, a 111-bp fragment of the ctrA gene was amplified using primers described by Corless *et al.*[220] Dual hybridisation probes with sequences of 5' TTCGTACTACATTGCCACGTGTCAGC-fluorescein 3' and 5' LCRed640-CACATTCG-TATCCTGCACATTTGCC-phosphate (Tib Molbiol, Berlin, Germany) were used for detection. The 20-µL reaction contained 8 µL of sample DNA, 1 x FastStart DNA Master Hybridisation Probes solution (Roche Diagnostics, Mannheim, Germany), 4mM MgCl2, 0.5 µM of both primers and 0.2 µM of both hybridisation probes. The real-time PCR assay was completed in a LightCycler Instrument (Roche Diagnostics), consisting of 10 min at 95°C followed by 50 cycles of 95°C for 10 s, 55°C for 10 s, and 72°C for 5 s. Every seventh specimen was a no-template control. A standard curve for quantification was included in each run, created by amplifying a tenfold dilution series of purified meningococcal DNA. Because of the poor precision in samples with low levels of the target DNAs, we gave all aetiologically proven samples with a PCR genome count <5 genomes/µL a predefined value of 0.5 genomes/µL. These analyses were conducted at the Finnish Institute for Health and Welfare (Oulu, Finland).

4.2.2.4 IDS-iSYS analyser (study V)

The serum 25-OHD concentrations were measured in Sture Andersson's laboratory (Children's Hospital, Pediatric Research Center, University of Helsinki, Helsinki University Hospital, Helsinki, Finland), using the automated immunoassay IDS-iSYS system (Immunodiagnostic Systems, Tyne and Wear, UK) according to the manufacturer's instructions. The IDS-iSYS system is based on chemiluminescence technology with acridinium as the chemiluminescent label, employing a competitive immunoassay design.

Based on an in-house comparison of 67 samples, the results obtained with the IDS-iSYS system agreed well and linearly with the results from liquid chromatography in tandem with mass spectrometry (R2 = 0.942). Compared to the National Institute of Standards and Technology standards, IDS-iSYS has shown a positive bias around 10%.[221] The manufacturer reports intra- and interassay coefficients of variation of 4% to 7% and 6% to 12 %, respectively, and a detection limit of 6.5 nmol/L.

4.2.3 Statistical methods

The statistical analyses for studies II through V were conducted using Statview software, version 5.0.1 (SAS institute, Cary, North Carolina, USA) and IBM's SPSS Statistics software, version 24 (IBM Corp., New York, USA). However, the local regression analysis for study II was performed in R, version 3.4.2 (R Foundation for Statistical Computing, Vienna, Austria).

First, the normality of the variables was visually inspected. All of the studies dealt with roughly nonnormally distributed variables and, consequently, nonparametric tests were primarily employed. The associations of the variables analysed to continuous patient characteristics were assessed using Spearman's rank correlation, whilst their relationship to categorical variables was estimated using the Mann–Whitney U test or the Kruskal–Wallis test. The chi-square test was employed when comparing categorical variables, and the Wilcoxon signed-rank test was used to assess differences in paired samples. In study V, the mean difference in the S-25-OHD concen-

tration between survivors and nonsurvivors and the 95% CIs were obtained using a t-test. Multivariate analyses were conducted using multiple linear regression analysis. If needed, the Bonferroni correction was applied in multiple comparisons. We considered $p < 0.05$ statistically significant.

When appropriate, the prognostic value of the variables studied was estimated using binary logistic regression analysis. In studies II through IV, median cut-off values for the variables were used, both to facilitate the interpretation of the results and to assess the wide distribution of the variables. Results are presented as odds ratios (ORs) with 95% CIs.

5 RESULTS

5.1 PROSPECTIVE, RANDOMISED CONTROLLED TRIAL (STUDY I)

5.1.1 Study design and patient characteristics

During the 5-year period, a total of 1128 children were assessed for eligibility. Amongst these, 375 fulfilled the requirements for study inclusion and were randomised to one of the treatment groups. Because two participants died before initiating treatment, the intention-to-treat population comprised 373 participants: 187 in the intervention group and 186 in the control group.

In both study groups, roughly 10% did not receive the allocated treatment. The most common reason for this was an inability to take oral medications due to severe illness. Other reasons included the administration of nonprotocol antibiotic treatments and sporadic failures in treatment because of practical or unknown reasons. Thus, 330 participants received the allocated treatment. However, a further 126 participants were excluded from the final per-protocol analysis, primarily because they did not fulfil our strict criteria for confirmed bacterial meningitis. Figure 15 provides a flowchart of the patient population.

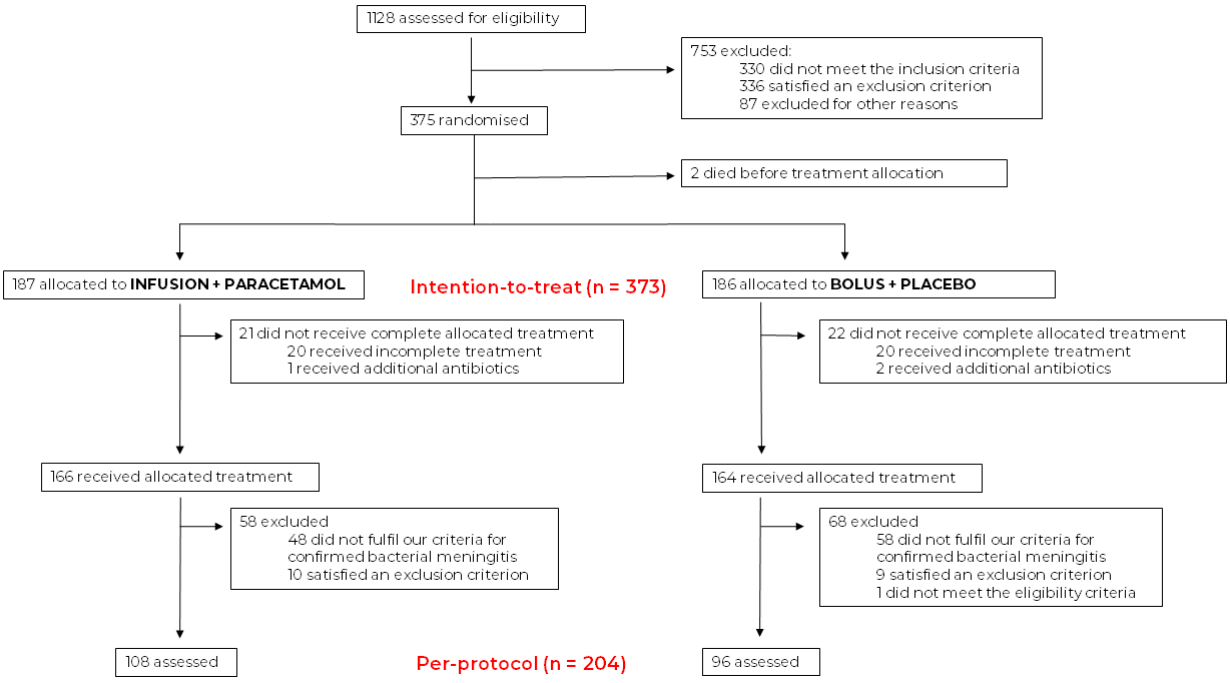

Figure 15 Flowchart of patient inclusion. Modified from the original manuscript.

Before initiating treatment, both study groups displayed similar baseline characteristics. Many participants were severely ill already upon admission: roughly one-third showed focal neurological signs in the emergency department and almost two-thirds suffered from respiratory distress, a sign of a severe overall condition. To support this, the median GCS score was 12 upon arrival. Malnutrition was prevalent, whereby 26% (96 / 371) of children had a weight-for-age z-score under -2 standard deviations (SDs) based on the WHO reference.[222] An HIV test was performed for roughly half of the children, for whom 8% (15 / 191) tested positive.

The causative bacterium was identified in 42% of participants, likely reflecting the fact that half of the patients had received prehospital antibiotics. *S. pneumoniae* was identified in 73 cases, *N. meningitidis* in 37 cases, Hib in 7 cases and some other bacteria in 39 cases.

The overall outcome of participants was gloomy. Upon discharge, 39% had died, 47% either died or survived with severe neurological sequelae and only 46% of participants survived without any neurological sequelae. At day 7 from treatment initiation, the overall mortality rate was 34%.

5.1.2 Effect of the treatment studied

The use of a continuous cefotaxime infusion combined with oral paracetamol for four days did not improve the outcomes from bacterial meningitis in these children when compared to conventional bolus treatment without paracetamol. Table 3 presents the impact of the treatment modality on day 7 mortality separately for the intention-to-treat and per-protocol populations. Furthermore, no differences emerged between the study groups regarding the time of death following initiating treatment, shown in Figure 16 for the per-protocol population.

In accordance with the study protocol, several subgroups were analysed separately in order to detect any potential differences in the treatment efficacy between children with differing baseline characteristics. The treatment effect remained similar across the subgroups. However, the overall mortality varied largely between different subgroups: an especially poor presenting clinical condition and a long duration of preadmission illness appeared to predict a worse disease outcome (Table 4).

Table 3. Impact of treatment modality on day 7 mortality

Outcome	Infusion + paracetamol, % (n)	Bolus + placebo, % (n)	Risk ratio (95 % CI)	Absolute risk difference, % (95% CI)
Day 7 death (ITT)	32.6 (61 / 187)	34.4 (64 / 186)	0.95 (0.71–1.26)	1.8 (-7.8 to 11.4)
Day 7 death (PP)	30.6 (33 / 108)	34.4 (33 / 96)	0.89 (0.60–1.32)	3.8 (-9.1 to 16.7)

Abbreviations: CI, confidence interval; ITT, intention-to-treat; PP, per-protocol. Modified from the original manuscript.

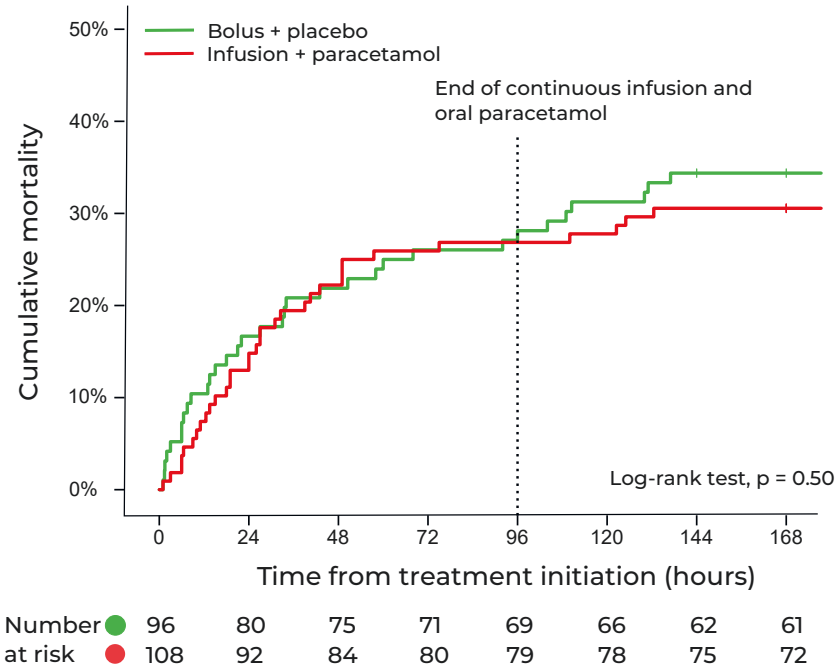

Figure 16 Mortality curves in the per-protocol population for both treatment groups. Ticks indicate censored participants. Modified from the original manuscript.

Table 4. Day 7 mortality in different patient subgroups

Variable	Mortality, % (n)	P value
Age		
Age < 12 months (n = 107)	34% (36 / 107)	0.94
Age ≥ 12 months (n = 265)	33% (88 / 265)	
Presenting condition[a]		
GCS < 12 (n = 178)	49% (87 / 178)	< 0.001
GCS ≥ 12 (n = 181)	18% (33 / 181)	
Duration of illness before hospital admission[b]		
≤3 days (n = 113)	20% (23 / 113)	< 0.001
>3 days (n = 252)	39% (98 / 252)	
Weight-for-age Z-score[c]		
Slightly malnourished or normal (≥-2 SDs) (n = 275)	30% (83 / 275)	0.04
Severely malnourished (<-2 SDs) (n = 96)	42% (40 / 96)	
Bacterial aetiology		
S. pneumoniae (n = 73)	40% (29 / 73)	0.21
Nonpneumococcal or unknown (n = 300)	32% (96 / 300)	

[a] Data on the GCS score were missing for 14 participants
[b] Duration of illness unknown for 8 participants
[c] Information missing for 2 participants
P values were obtained using a chi-square test. Modified from the original manuscript

5.2 RETROSPECTIVE COHORT STUDIES (STUDIES II–V)

5.2.1 Matrix metalloproteinases (study II)

The levels of MMP-8, MMP-9, TIMP-1 and MPO in the CSF of children with bacterial meningitis were examined in a sample of 245 children from Latin America (Table 5). To identify any potential differences between these inflammatory mediators, we first looked for correlations between our results and baseline patient characteristics. We noted that MMP-8 and MPO seemed to exhibit rather similar relationships to the baseline characteristics we studied; specifically both correlated with the CSF protein level and CSF white cell count, but not with clinical variables such as bacterial aetiology or illness duration. In contrast, MMP-9 did not correlate with the basic CSF parameters, but differed in terms of the causative agent. Pneumococcal meningitis induced the highest MMP-9 levels in the CSF, although the only significant difference in pairwise comparisons emerged between *S. pneumoniae* and Hib ($p = 0.001$).

We then constructed a correlation matrix with our results, in an attempt to illustrate the interaction between these mediators.

Table 5. Main characteristics of children included in studies II–V

Variable	Study II	Study III	Study IV	Study V
Research focus	**CSF matrix metalloproteinases**	**CSF cathelicidin**	**CSF cathelicidin vs. bacterial load**	**Serum vitamin D**
Patients, n	245	99	76	142
Age in months, mean (SD)	25 (40)	23 (39)	25 (39)	15 (26)
Male sex, n (%)	140 / 245 (57.1)	54 / 99 (54.5)	52 / 76 (68.4)	84 / 142 (59.2)
GCS upon admission, mean (SD)	12 (3)	13 (2)	11 (3)	12 (3)
Duration of illness in days, mean (SD)	2 (2)	2 (2)	3 (3)	3 (3)
Bacterial aetiology				
H. influenzae, n (%)	122 (49.8)	40 (40.4)	44 (57.9)	81 (57.0)
S. pneumoniae, n (%)	64 (26.1)	26 (26.3)	28 (36.8)	43 (30.3)
N. meningitidis, n (%)	10 (4.1)	7 (7.1)	4 (5.3)	9 (6.3)
Other, n (%)	12 (4.9)	6 (6.1)	0 (0)	9 (6.3)
Unknown, n (%)	37 (15.1)	20 (20.2)	0 (0)	0 (0)
Disease outcomes				
Mortality, n (%)	33 / 245 (13.5)	14 / 99 (14.1)	9 / 76 (11.8)	23 / 142 (16.2)
Deafness, n (%)	30 / 165 (18.2)	13 / 73 (17.8)	11 / 51 (21.6)	22 / 98 (22.4)
Death or SeNeSe, n (%)	58 / 242 (24.0)	17 / 98 (17.3)	19 / 74 (25.7)	35 / 139 (25.2)
Death or AnNeSe, n (%)	112 / 241 (46.5)	36 / 98 (36.7)	33 / 74 (44.6)	64 / 139 (46.0)

Abbreviations: AnNeSe, any neurological sequelae; SeNeSe, severe neurological sequelae

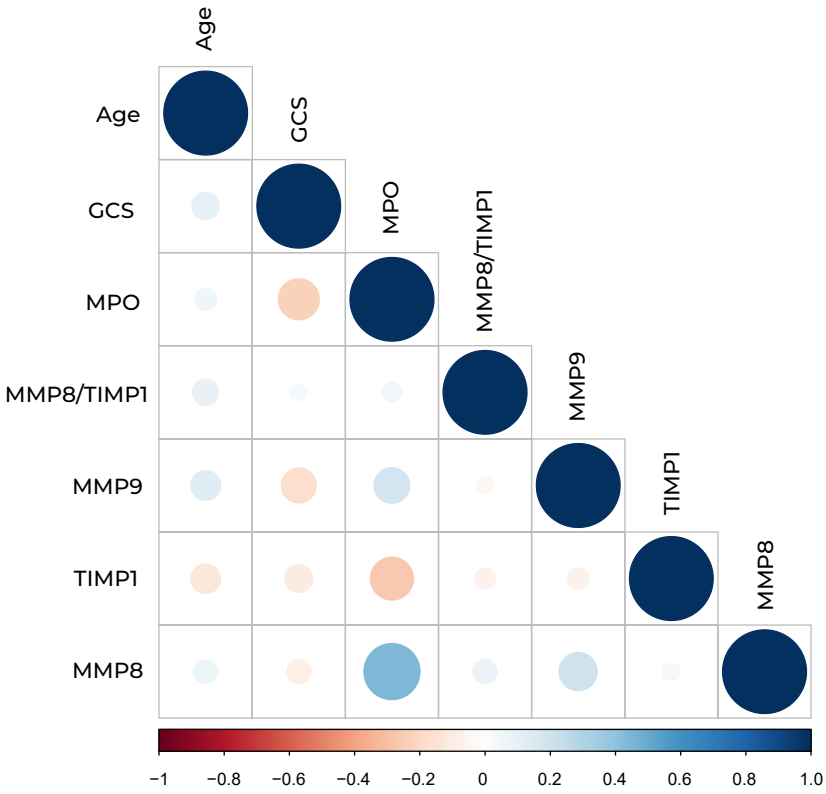

Figure 17 Correlation matrix of the molecules studied.

These results are presented visually in Figure 17. As expected, MPO correlated positively with MMP-8 and MMP-9, although the correlation between MPO and MMP-9 seemed less strong than that for with MMP-8. A weak correlation existed between MMP-8 and MMP-9. Finally, TIMP-1 exhibited a clear negative correlation with MPO, but seemed unrelated to MMP-8 or MMP-9.

Lastly, we investigated the extent to which the CSF levels of these inflammatory mediators predicted the outcomes of bacterial meningitis. In addition to the crude ORs, we also calculated the ORs following adjustment for the clinical condition upon admission. This adjustment was considered necessary, since the presenting clinical condition appears crucial for disease prognosis.[149]

Table 6 presents the adjusted ORs separately for death and for the composite outcomes of death or severe neurological sequelae and death or any neurological sequelae. The median cut-off value of the CSF MMP-8 concentration emerged as the best prognostic marker, especially in terms of mortality: a CSF MMP-8 concentration above the median value increased the odds of death 4.9-fold. When including neurological deficits to the outcome, the adjusted ORs for the median cut-off MMP-8 value decreased to 2.3 and 2.2. Turning to the other inflammatory mediators, high CSF levels for both MPO and the molar ratio of MMP-8-to-TIMP-1 pre-

Table 6. Adjusted odds ratios (95% CI) for disease outcomes. Calculated for concentrations above the median value and adjusted for the clinical condition upon admission.

Variable	Death	Death or severe neurological sequelae	Death or any neurological sequelae
MMP-8	4.9 (1.8–12.9)	2.3 (1.2–4.6)	2.2 (1.2–4.0)
MPO	2.0 (0.8–4.8)	2.2 (1.1–4.4)	1.6 (0.9–2.8)
Total MMP-9	1.4 (0.6–3.3)	0.9 (0.5–1.8)	1.2 (0.7–2.2)
TIMP-1	0.7 (0.3–1.6)	0.8 (0.4–1.5)	1.2 (0.7–2.2)
MMP-8/ TIMP-1	2.8 (1.2–7.0)	1.3 (0.6–2.5)	1.1 (0.6–2.0)

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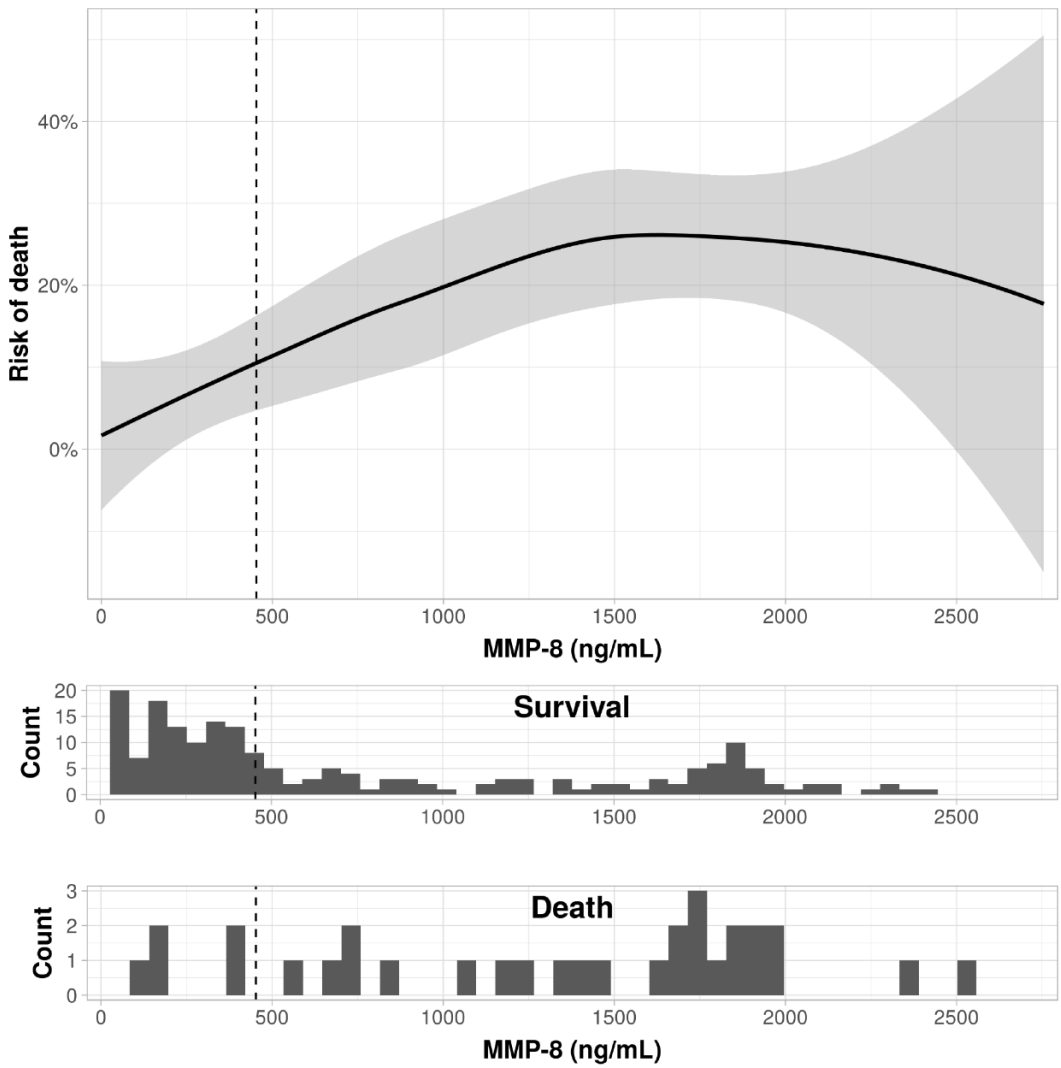

Figure 18 The estimated risk of death for different concentrations of CSF MMP-8. The vertical dashed line represents the median CSF MMP-8 concentration, whilst the area shaded in grey depicts the 95% confidence interval. Calculated using local regression. Modified from the original manuscript.

dicted poor disease outcomes to a minor degree. Figure 18 shows the risk of death as a function of the CSF MMP-8 concentration.

5.2.2 Cathelicidin (studies III & IV)

The role of CSF cathelicidin in the inflammatory cascade triggered by bacterial meningitis was examined in two separate studies. Study III focused on the dynamics of the CSF cathelicidin expression, whilst study IV assessed the relationship between the CSF cathelicidin and the CSF bacterial burden. These studies comprised different patient cohorts, since study IV included only patients in whom the CSF bacterial genome count was previously analysed.[219] Table 5 (above) summarises the patient characteristics for these cohorts.

The dynamics of the CSF cathelicidin expression were investigated by measuring the CSF cathelicidin concentration in paired CSF samples, obtained upon admission and 12 to 24 hours later. Overall, the cathelicidin concentration in these samples varied from undetectable to 1258 ng/mL. The median cathelicidin concentrations were 27.9 ng/mL (interquartile range [IQR] 5.1–98.6) in the CSF sample obtained on admission (CSF1, n = 89) and 9.5 ng/mL (IQR 3.4–46.8) in the sample obtained 12 to 24 hours later (CSF2, n = 77), a statistically significant difference (p = 0.006). The CSF cathelicidin concentration did not differ according to the causative bacteria or patient age, but correlated positively with the CSF white cell count (CSF1, rho 0.528, p < 0.001; CSF2, rho 0.206, p = 0.12) and protein level (CSF1, rho 0.618, p < 0.001; CSF2, rho 0.466, p < 0.001). In terms of the clinical presentation, patients with a lower GCS score upon admission tended to exhibit higher levels of CSF cathelicidin, especially after treatment initiation (rho -0.301, p = 0.01). Yet, the cathelicidin level upon admission negatively correlated with the duration of the prehospital illness in days: the longer the patient had suffered from disease, the lower the cathelicidin level in the CSF (rho -0.472, p = 0.004).

In terms of prognostics, the CSF cathelicidin levels above the median upon admission or after treatment initiation did not predict disease outcome in terms of death or the composite outcomes of death or any or severe neurological sequelae (Table 7). However, patients with an optimal GOS score (5 / 5) had higher initial CSF cathelicidin concentrations than those with a GOS score <5 (median concentrations of 36.2 ng/mL

Table 7. Adjusted odds ratios (95% CI) for different disease outcomes. Calculated for concentrations falling above the median value and adjusted for the clinical condition upon admission.

Variable	Death	Death or severe neurological sequelae	Death or any neurological sequelae
CSF1 Cathelicidin	0.6 (0.15–2.3)	0.8 (0.2–2.7)	0.5 (0.1–1.4)
CSF2 Cathelicidin	2.6 (0.4–18.0)	4.4 (0.7–26.7)	0.7 (0.2–2.4)

Abbreviations: CSF1, CSF sample obtained upon admission; CSF2, CSF sample obtained 12–24 hours after treatment initiation.

[IQR 10.5–146.1] vs. 9.9 ng/mL [IQR 3.5–67.8], p = 0.026).

In order to evaluate the variation in the CSF cathelicidin expression in response to treatment, we calculated the ratio of CSF2-to-CSF1 cathelicidin, reflecting the relative change in the CSF cathelicidin concentration after initiating treatment. The ratio of CSF2-to-CSF1 cathelicidin negatively correlated with the GOS score at discharge (rho -0.278, p = 0.026): that is, the smaller the ratio, the better the outcome. Similarly, a decreasing CSF cathelicidin concentration during the first 12 to 24 hours related to greater odds of an optimal GOS score (OR 3.2, 95% CI 1.1–9.4) when compared to increasing or stable CSF cathelicidin levels.

5.2.2.1 Relationship between CSF cathelicidin and CSF bacterial genome count (study IV)

In this series, the median CSF cathelicidin values were 33.5 ng/mL (IQR 10.5–122.2) for CSF1 and 1.5 ng/mL (IQR 0.3–5.4) for CSF2. In contrast to our previous study, the CSF cathelicidin concentrations here neither associated with the CSF white cell counts nor with the clinical condition upon admission.

We detected a notable correlation between the cathelicidin concentration and the bacterial genome count in the CSF samples obtained both upon admission (n = 65; rho 0.53, p < 0.0001; Figure 19) and 12 to 24 hours after initiating treatment (n = 40; rho

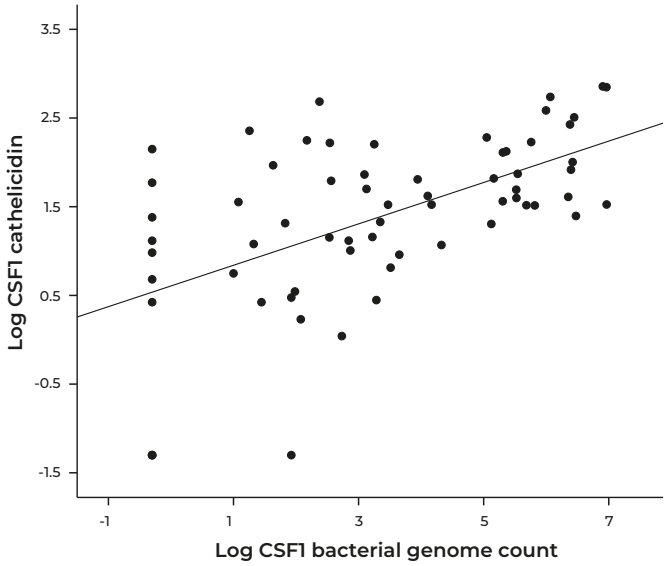

Figure 19 Regression plot of the cathelicidin concentration versus the bacterial genome count in the initial CSF sample (n = 65, R^2 = 0.313). Modified from the original manuscript.

Table 8. Odds of an above the median ratio of the CSF1 cathelicidin-to-bacterial genome count vs. different outcomes. Adjusted for the clinical condition upon admission.

Outcome	n	OR	95 % CI	P value
Death	64	0.92	0.16–5.22	0.92
Death or SeNeSe	62	0.57	0.16–2.07	0.39
Death or AnNeSe	62	0.26	0.08–0.81	0.02
SeNeSe	53	0.51	0.10–2.63	0.42
AnNeSe	53	0.22	0.06–0.79	0.02
Deafness	40	0.13	0.02–0.69	0.02
Any hearing impairment	40	0.13	0.02–0.71	0.02
Optimal GOS score*	54	3.04	0.89–10.3	0.07

*GOS score of 5. Abbreviations: AnNeSe, any neurological sequelae; CSF1, CSF sample obtained upon admission; GOS, Glasgow Outcome Scale; SeNeSe, severe neurological sequelae. Modified from the original manuscript.

0.553, p = 0.0006). In fact, amongst all of the variables analysed, the CSF cathelicidin most strongly correlated with bacterial load. Interestingly, we also found that a high initial cathelicidin-to-genome count ratio associated with a better disease prognosis: we identified a positive correlation between this ratio and both the overall prognosis in terms of the GOS score (n = 55; rho 0.354, p = 0.009) and the audiological outcome assessed as hearing (as an ordinal variable) in the better ear (n = 41; rho 0.407, p = 0.01). To further investigate this relationship, we calculated the odds of a cathelicidin-to-genome count ratio above the median value for various disease outcomes (Table 8). After adjusting for clinical condition upon admission, a high initial ratio of cathelicidin-to-bacterial genome count related to a better audiological outcome and to fewer neurological sequelae.

5.2.3 Vitamin D (study V)

Study V comprised a cohort of 142 children, originating from the Dominican Republic (n = 85), Ecuador (n = 41) and Venezuela (n = 16). Table 5 (above) summarises the patient characteristics.

The median S-25-OHD concentration was 96 nmol/L, ranging from 19 to 251 nmol/L. Deficient levels (<50 nmol/L) were detected in 5 children (4%), whereas 31 children (22%) showed an excessive S-25-OHD concentration (>125 nmol/L). A young age, low weight-for-age Z-scores and low initial blood glucose values all associated with an S-25-OHD deficiency.

When assessed as a continuous variable, S-25-OHD associated with the CSF glucose and serum sodium levels upon admission, as well as with the country of origin. However, only the country of origin remained an independent predictor of the S-25-OHD concentration in a multivariate model: children from Ecuador exhibited significantly lower levels of S-25-OHD than children from the Dominican Republic or Venezuela (p < 0.001).

A child's S-25-OHD level did not associate, however, with survival (the mean S-25-OHD concentrations in survivors and nonsurvivors were 102.2 nmol/L and 105.9 nmol/L, respectively; mean difference -3.7

nmol/L, 95% CI for the difference -23.9–16.5), severe neurological sequelae, deafness or with the overall outcome in terms of the GOS score at discharge. However, children who survived and experienced no neurological sequelae showed slightly higher S-25-OHD concentrations than those who experienced any kind of neurological sequelae (median S-25-OHD concentrations of 101 nmol/L [IQR 85–127] vs. 89 nmol/L [IQR 75–111], p = 0.04). By contrast, higher S-25-OHD levels tended to associate with a greater likelihood of a hearing impairment: children with an S-25-OHD >75 nmol/L had a worse audiological outcome in terms of the hearing in the better ear (as an ordinal variable), compared to those with S-25-OHD <75 nmol/L (median scores of 4 [IQR 3–5] vs. 5 [IQR 5–5], p = 0.01). However, this association was no longer significant when the more exact combined hearing threshold in both ears was used (median thresholds of 105 dB [IQR 70–166] and 80 dB [IQR 50–121], respectively, p = 0.07).

Finally, S-25-OHD was plotted against the CSF cathelicidin concentrations, available from 115 samples taken upon admission (CSF1, median concentration 35.4 ng/mL, IQR 9.1–104.7) and from 76 samples taken 12 to 24 hours after initiating treatment (CSF2, median concentration 7.6 ng/mL, IQR 3.3–45.5). No correlation was detected between the S-25-OHD and the CSF cathelicidin concentration in either sample (CSF1, rho -0.071, p = 0.45; CSF2, rho 0.010, p = 0.93; Figure 20).

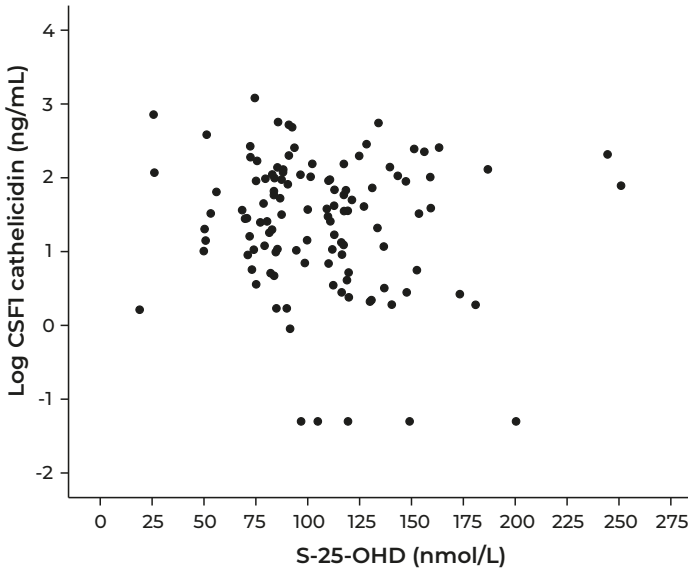

Figure 20 S-25-OHD and CSF1 cathelicidin concentrations (n = 115). Rho -0.071, p = 0.45. Abbreviations: CSF1, CSF sample obtained upon admission; S-25-OHD, serum 25-hydroxyvitamin D.

6 DISCUSSION

6.1 CONTINUOUS CEFOTAXIME INFUSION AND ORAL PARACETAMOL AS TREATMENT FOR BACTERIAL MENINGITIS

Our double-blind, randomised parallel-group study comprising 373 children identified no benefit from using a continuous 4-day cefotaxime infusion combined with oral paracetamol as treatment for bacterial meningitis in Angola. Neither mortality alone nor the composite outcomes of death or severe or any neurological sequelae differed between those children who received our modified treatment and those treated conventionally with antibiotic boluses. Yet, our treatment intervention caused no detectable harm either.

Beyond the supporting theoretical rationale, the definitive motive for the setup of this trial arose from a previous study conducted by our research group, carried out at the same hospital in Luanda.[83] In that study, continuous cefotaxime infusion for 24 hours combined with oral paracetamol for 48 hours dramatically improved the initial survival of children (Figure 10, page 42). Although statistical significance was lost upon discharge from hospital, the benefit of the modified regimen seemed rather distinct and prompted the setup of a follow-up study with a longer intervention. We hypothesised that extending this treatment modality to four days would result in a further improvement of the prognosis; instead, we detected no benefit from the intervention. What might explain this discrepancy?

Whilst we lack a plausible explanation, certain differences, which might contribute to this disparity, were eventually detected between the studies. Table 9 provides a comparison of baseline patient characteristics. Briefly, participants in the second study seemed even more ill when presenting, had received more prehospital antibiotics and were older than the participants of the first study. In addition, the aetiological distribution shifted: the new study comprised only a few cases of Hib meningitis, but consequently more children with less common meningitis pathogens and a larger proportion with an unknown bacterial aetiology.

We hypothesise that one major factor contributing to the lack of a treatment effect in the current study might be the participants' desperate clinical condition upon presentation at hospital. Because nearly one-third of the patients presented with focal neurological signs, suggestive of already established intracranial complications, many of these children may simply have passed the point when our intervention might have been beneficial. In a recent review,[114] Nau *et al.* present a similar hypothesis regarding the adjuvant therapy of bacterial meningitis based on their experimental studies. Figure 21, modified from that review, illustrates the hypothetical relationship between the duration or stage of illness and the risk of adverse outcomes, illustrating how the potential of an adjuvant treatment is likely restricted to a specific time period. Presuming that the participants in our study were frequently situated on the right-hand side of the therapeutic window shown in Figure

Table 9. Baseline patient characteristics of the two infusion studies in Luanda

Characteristic	Previous study	Current study	P value
Age in months (IQR)	14 (7–42)	28 (9–70)	<0.0001
Male sex (%)	366 / 723 (50.6)	216 / 373 (57.9)	0.15
Duration of illness, days (IQR)	5 (3–7)	5 (3–8)	0.0078
Previous antibiotics (%)	271 / 679 (39.9)	168 / 335 (50.1)	0.002
GCS score (IQR)	12 (8–15)	12 (9–15)	0.11
Altered consciousness (%)	499 / 723 (69.0)	262 / 357 (73.4)	0.14
Signs of malnutrition (%)	111 / 723 (15.4)	91 / 369 (24.7)	0.0002
Signs of dehydration (%)	116 / 723 (16.0)	89 / 365 (24.4)	0.0009
Focal neurological signs (%)	173 / 723 (23.9)	113 / 357 (31.7)	0.0068
Dyspnoea (%)	333 / 723 (46.1)	211 / 358 (58.9)	<0.0001
Other focus of infection (%)	182 / 723 (25.2)	108 / 347 (31.1)	0.04
Bacterial aetiology			
S. pneumoniae (%)	184 / 723 (25.4)	73 / 373 (19.6)	0.15
N. meningitidis (%)	49 / 723 (6.8)	37 / 373 (9.9)	0.33
H. influenzae (%)	188 / 723 (26.0)	7 / 373 (1.9)	<0.0001
Other bacteria (%)	32 / 723 (4.4)	39 / 373 (10.5)	0.0005
Unknown (%)	270 / 723 (37.3)	217 / 373 (58.2)	<0.0001

Data are presented as number of patients (%), unless otherwise indicated. P values were obtained using a chi-square test or the Mann–Whitney U test. Bonferroni's correction was applied in comparisons of different aetiologies. Modified from the original manuscript.

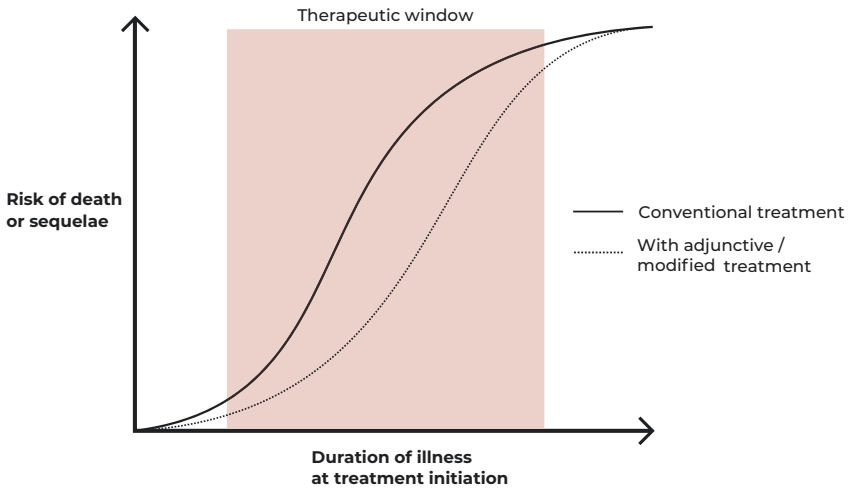

Figure 21 Relationship between the duration of illness and the risk of adverse disease outcomes, and the hypothesised effect of modified treatment. Modified from Nau *et al.*[114]

21, this might partly explain the poor treatment effect. To support this, we found that the clinical condition upon arrival to hospital associated with the mortality rate: the sicker the patient upon admission, the poorer the prognosis (Table 4, page 63). This observation also mirrors previous studies.[148,149]

The frequent use of prehospital antibiotics in our series is another factor possibly contributing to this finding. The rationale for using an initial continuous β-lactam infusion in bacterial meningitis relies, at least partly, on attenuating the inflammatory burst triggered by the bacteriolytic antibiotic in the CSF. However, because half of the participants had received antibiotics, often for several days before presenting at hospital, the bacteriolysis in the CSF was likely already triggered before hospital admission.

The potential impact of the shift in bacterial aetiology remains intriguing. Because the current study included proportionally more children with infections caused by less common meningitis pathogens or with an unknown bacterial aetiology, this raises a question regarding whether the intervention might be less beneficial in such cases. Comparing these two groups with the remainder of the sample, we detected no differences in the treatment effect, supporting such a hypothesis. The disappearance of Hib meningitis, however, may also have played a role.

Furthermore, we did not reach our target of 165 participants with confirmed bacterial meningitis in both treatment arms, primarily due to the large number of subjects excluded from the final per-protocol population. Amongst others, those excluded comprised patients with viral or tuberculous infections, and likely also included children with partially treated bacterial meningitis. The drop-out rate at this point was slightly higher than in our previous trial and, most importantly, was not properly taken into account when calculating the sample size of the follow-up trial.[83] If we look only at the per-protocol population, the intervention effect, indeed, seemed slightly more promising with a similar tendency noted in children with pneumococcal disease when compared to other or unknown bacterial aetiology. However, both these analyses are flawed given the lack of statistical power, barring us from making more definitive conclusions based on these results.

Turning to studies on a continuous β-lactam infusion in other severe infections, findings seem similarly controversial. The use of a continuous infusion in critically ill adult patients resulted in better clinical outcomes in some studies,[161,163] whilst others found no benefit.[162] It seems that the optimal population to benefit from this intervention remains unclear, although severely ill patients with infections caused by less susceptible Gram-negative bacteria might represent the most promising target population.[223] In light of the previous discussion, the duration of illness and the stage of infection should be taken into account when further evaluating the potential of this dose regimen.

To conclude, our study in Angola identified no benefit from the use of a continuous β-lactam infusion combined with oral paracetamol as treatment for bacterial meningitis in children. The high mortality rate in our study mirrors previous findings from the same setting,[83] reflecting the desperate situation that continues to prevail in this region. In order to enable prompt and adequate initiation of treatment, improving the availability of healthcare services as well as improving the parental knowledge of this disease seem vital to ameliorating prognosis for bacterial meningitis in these settings. In terms of prevention, large-scale immunisations against the relevant bacteria are obviously warranted.

6.2 PREDICTIVE FACTORS IN THE CSF

In our retrospective studies, we intended to clarify the role of MMP-8 and MMP-9, their inhibitor TIMP-1, their activator MPO and the antimicrobial protein cathelicidin as part of the inflammatory cascade in the CSF during bacterial meningitis. In particular, we focused on the potential prognostic value of these markers, intending to identify new diagnostic tools and future targets for adjuvant treatment.

Overall, MMP-8 emerged as the most promising prognostic marker for bacterial meningitis. A low level of CSF MMP-8 associated with a higher probability of survival as well as with a lower risk of neurological sequelae, whilst high concentrations predicted poorer outcomes.

Unfortunately, our study does not pinpoint the pathways via which high levels of CSF MMP-8 associate with a poor prognosis. Previous experimental studies suggest that MMP-8 is involved in the degradation of the blood–brain barrier and promotes neutrophil recruitment during infection.[170,179] Thus, it seems likely that MMP-8 contributes to the inflammatory burst in the CSF during bacterial meningitis. Because the strength of this inflammatory reaction has been repeatedly associated with poor disease outcomes, the results of our study appear to agree with current understandings of meningitis pathophysiology.

Previously, only two clinical studies investigated the expression of MMP-8 in the CSF during meningitis. Both studies reported median concentrations of approximately 100 ng/mL, extending to >1000 ng/mL.[183,185] The median MMP-8 concentration was roughly fourfold higher in our study, yet the patients included in our study also experienced worse disease outcomes. The study by Leppert *et al.*[185] also examined the association between the MMP-8 concentration and neurological sequelae, finding no relationship between these variables. However, their small sample size (n = 27) might contribute to this discrepancy.

Furthermore, we intended to clarify the relationship between MPO and MMP-8, MMP-9 and TIMP-1 in the CSF during bacterial meningitis. This approach stemmed from previous experimental work, suggesting that MPO can both activate MMPs and inactivate TIMPs.[64,65,224] Indeed, we found that the higher the concentration of MPO, the higher the levels of MMP-8 and MMP-9, whilst the relationship between MPO and TIMP-1 was inverse (Figure 17, page 65). However, our findings represent a momentary correlation between these variables, not necessarily indicating causality. Considering the role of these mediators during inflammatory reactions, it seems probable that MPO and these MMPs are upregulated via similar pathways, possibly explaining our finding.

The predictive value of CSF cathelicidin in children with bacterial meningitis was less distinct, whereby no relationship was detected between the CSF cathelicidin levels and the outcome in terms of mortality or the composite outcomes of death or severe or any neurological sequelae. A high CSF cathelicidin concentration upon admission associated with an optimal recovery in terms of the GOS score, although this finding is confounded by the fact that the CSF cathelicidin levels were higher in children who presented earlier at hospital. Moreover, a decreasing CSF cathelicidin level following treatment initiation associated with an optimal recovery, possibly reflecting a

diminishing inflammatory reaction resulting from successful treatment. Increasing CSF cathelicidin levels might again reflect a further strengthening inflammatory burst, leading to the excessive production of cytotoxic substances and, ultimately, to parenchymal damage.[52]

Only a few previous studies exist that addressed the CSF cathelicidin level in patients with bacterial meningitis. In a sample of eight adult meningitis patients, Brandenburg *et al.* reported a mean CSF cathelicidin concentration of 84.1 ng/mL.[62] Another study on childhood tuberculous meningitis used a combination of viral meningitis (n = 25), bacterial meningitis (n = 10) and nonmeningitis (n = 20) cases as the control group, for whom the CSF cathelicidin concentrations fell between roughly 0.003 ng/mL and 10 ng/mL.[197] Given that our study also found CSF cathelicidin levels varying over a thousandfold without a clear explanation, many details regarding the expression of CSF cathelicidin remain unclear.

Whilst we detected an association between the CSF white cell count and the cathelicidin level in our first cathelicidin study (study III), such a relationship remained undetected in the latter study that assessed the relationship between CSF cathelicidin and the bacterial burden (study IV). Comparing these two patient cohorts reveals certain differences in baseline characteristics: the participants in study IV presented at hospital later during the disease, and often in a worse clinical condition than those in study III (Table 5, page 64, Mann-Whitney U test, p = 0.05 and p = 0.006, respectively). Furthermore, the aetiological distribution differed, given that roughly one-fifth of participants in study III had an unknown aetiology. Acknowledging that the concentration of CSF cathelicidin during meningitis appears to rapidly change, these differences in patient characteristics might contribute to this discrepancy between our studies.

Study IV aimed to deepen our understanding of cathelicidin in meningitis by investigating its relationship to the bacterial load in the CSF. The strong correlation between CSF cathelicidin and the bacterial load, particularly in the absence of a concomitant correlation between CSF cathelicidin and the white cell count, was somewhat unexpected. The cathelicidin response in the CSF appears closely linked to the local bacterial burden, but not exclusively mediated by the CSF leukocytes; indeed, resident immune cells from the CNS and different epithelial cells likely also contribute to the CSF cathelicidin level during meningitis.[62,193,198] In fact, previous reports indicated that the CSF white cell count poorly reflects the CSF bacterial load in meningitis.[219]

In study IV, we also considered the initial ratio of CSF cathelicidin to the bacterial load as a potential predictor of disease outcomes. In terms of neurological outcomes, this ratio did not appear to bring any additional prognostic value compared to the bacterial load alone. Whilst considering audiological outcomes, however, a high initial ratio of CSF cathelicidin-to-bacterial load seemed, in contrast to a low bacterial load alone, to associate with a better prognosis. This association was not detectable for CSF cathelicidin alone, suggesting that both the host's response (reflected by cathelicidin) and the stage of infection (as measured by the CSF bacterial load) affect the development of a hearing impairment in meningitis. In light of this and previous experimental work, surviving meningitis without audiological sequelae probably requires a well-balanced immune response sufficiently strong to restrain bac-

terial growth, but appropriately gentle so as not to induce collateral damage.[78]

When examining the CSF samples originating from the study in Latin America from 1996 through 2003, we must note that the samples were frozen for a rather long period before the current analyses were completed in 2015 through 2017, thus leading to questions regarding the stability of the CSF inflammatory mediators examined. By projecting the storage time (range from 143 to 219 months) against the concentrations of the studied molecules (CSF MMP-8, MMP-9, MPO, TIMP-1 and cathelicidin), we attempted to identify signs of degradation, finding no correlations (possibly indicating sample instability during storage). Moreover, the primary results of these retrospective studies focused on the differences of these mediators' concentrations within the same cohort, and not the absolute CSF concentrations. However, we cannot fully dismiss the possibility of minor degradation or inactivation of the studied molecules during storage.

More broadly, cathelicidin, MMP-8, MMP-9 and MPO seemingly contribute to the host's innate immune response in the CSF upon bacterial invasion. Although the cellular source of these compounds was not established in our studies, some part likely originates from different granules secreted by neutrophils.[225] Azurophilic granules, specific granules and gelatinase granules differ, except for their contents, also in terms of the propensity for exocytosis. Thus, the inflammatory mediators studied might contribute to different stages of the host's immune response to bacterial meningitis: whilst granules with MMP-9 are easily prone to secretion, granules with MMP-8 and cathelicidin or MPO require more stimuli for exocytosis (Figure 22).

To evaluate this hypothesis, a new correlation matrix was performed combining all data from studies II through IV (Table 10). Indeed, it appears as though CSF cathelicidin, MMP-8 and MPO rather closely correlate to with one other, whilst MMP-9 appears to

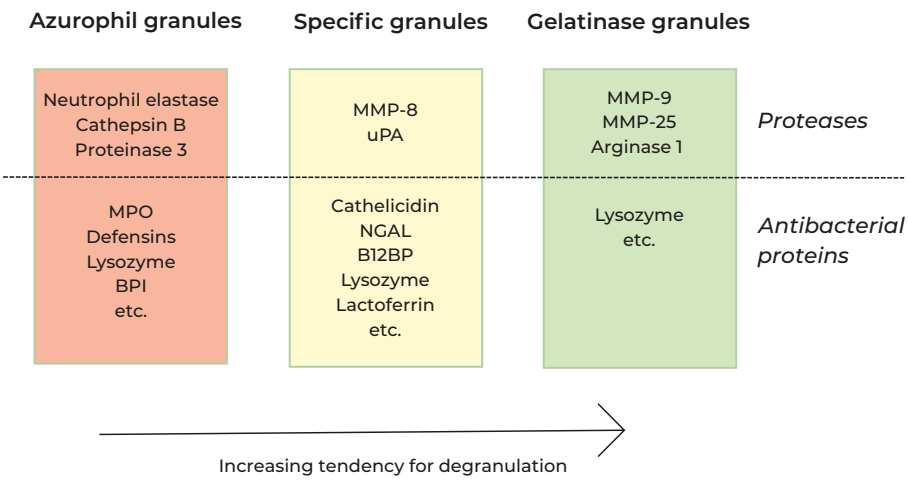

Figure 22 Selected characteristics of three different neutrophil granules. Abbreviations: B12BP, vitamin B12 binding protein; BPI, bactericidal/permeability-increasing protein; NGAL, neutrophil gelatinase-associated lipocalin; uPA, urokinase-type plasminogen activator. Modified from Serhan *et al.*[226]

Table 10. Correlation matrix for MPO, MMP-8, MMP-9 and cathelicidin in the CSF

Variable	MPO	MMP-8	Total MMP-9	Cathelicidin
MPO	N/A	ρ 0.496 p < 0.001	ρ 0.153 p = 0.02	0.692 p < 0.001
MMP-8	ρ 0.496 p < 0.001	N/A	ρ 0.160 p = 0.02	0.390 p < 0.001
Total MMP-9	ρ 0.153 p = 0.02	ρ 0.160 p = 0.02	N/A	0.163 p = 0.03
Cathelicidin	0.692 p < 0.001	0.390 p < 0.001	0.163 p = 0.03	N/A

Spearman's rank correlations.

differ from the others. However, this result might be confounded by differing laboratory methods.

In conclusion, our three explorative studies provide a glimpse of the complex inflammatory reaction that prevailing in the CSF during bacterial meningitis. This glimpse is both momentary and biased by the narrow perspective of our studies, but it provides novel information on the role of these inflammatory mediators in meningitis. Due to the retrospective nature of these studies, no causal relationships could obviously be substantiated, and the current results should be considered preliminary and hypothesis-generating in the absence of further confirmatory findings.

6.3 VITAMIN D, CATHELICIDIN AND MENINGITIS

The rationale for study V examining the impact of children's vitamin D status on outcomes of bacterial meningitis stemmed from an expanding body of evidence suggesting an immunomodulatory role for vitamin D.[204,205] However, we detected no association between vitamin D status upon admission and outcomes in terms of mortality or severe neurological impairment. We found that children who recovered without any neurological sequelae had a slightly higher vitamin D concentration upon admission compared to those who experienced any neurological sequelae. Yet, low vitamin D levels tended to associate with a better audiological outcome.

More generally, the vitamin D levels detected in our study were surprisingly high. The median S-25-OHD concentration in our cohort (96 nmol/l) appeared to be 20 to 30 nmol/l higher than previous reports from the same region.[227,228] However, the study by Checkley *et al.* comprised notably older children.[227] In our cohort, we noted that children from Ecuador showed lower S-25-OHD levels than children from the Dominican Republic or Venezuela. Differences in geographic conditions might contribute to this result, but also dietary habits and the use of vitamin D supplementation likely differ between the countries. Lastly, even with the most rigorous attention to high-quality sample storage, differing sample management

resulting in possible degradation cannot be completely dismissed.

The associations we detected in relation to both the audiological and the mild neurological sequelae remained rather weak. In particular the association between vitamin D levels <75 nmol/l and a better hearing outcome remains questionable, as we detected no significant relationship when including information from both ears. Whilst these are the first indications of an association between a child's vitamin D status and bacterial meningitis outcomes, our results warrant further study to explore this relationship more thoroughly. Because only five patients in our cohort presented with a vitamin D deficiency (S-25-OHD <50 nmol/l), our study did not definitely determine whether vitamin D deficiency impairs the host defence and, thus, bacterial meningitis outcomes.

We also examined whether the circulating vitamin D level associated with the cathelicidin concentration in the CSF. Previous studies on the relationship between S-25-OHD and plasma cathelicidin have yielded inconsistent results. [213–216] To our knowledge, however, our study represents the first to evaluate the hypothetical impact of S-25-OHD on the CSF cathelicidin levels. Our findings identified no association between these two variables, although this result again remains hampered by the lack of vitamin D-deficient subjects.

6.4 FUTURE PROSPECTS

The results from our clinical study in Angola were rather distinct. The use of a continuous infusion combined with oral paracetamol did not benefit children with bacterial meningitis in Angola, and further studies examining this regimen appear unwarranted, at least in similar settings. Nonetheless, the hopelessly poor outcomes from this series urgently call for the necessity of further interventions. Although large-scale vaccinations likely remain the most effective way to tackle meningitis, new treatment options should also be identified. Experimental studies suggest a benefit from using nonbacteriolytic antibiotics, although it remains unclear whether these results will translate into better clinical outcomes.

The difficulty of properly selecting participants to include in our clinical study in Angola, resulting in the exclusion of a large number of unconfirmed meningitis subjects from the final per-protocol population, reflects the challenging differential diagnostics of meningitis in such settings. Viral, parasitic, tuberculous and bacterial infections might present with similar symptoms, such as fever and a decreased level of consciousness. Given that diagnostic tools often remain scarce, this dilemma might lead to initially incorrect diagnoses, the delayed treatment initiation and, at worst, treatment failure and subsequent death.

To tackle this issue, we have enrolled patients in Angola to a prospective study assessing the aetiology of nontraumatic coma in children. We hope to clarify the underlying spectrum of illnesses and their distribution according to age, symptoms and findings upon admission, and to further describe the aetiological-specific outcomes.

Retrospective studies II through IV evaluated the expression of cathelicidin, MMP-8, MMP-9, MPO and TIMP-1 in the CSF during bacterial meningitis, clarifying the potential

prognostic role of these inflammatory mediators. In particular, the expression of the CSF MMP-8 and its association with disease outcomes seemed clear-cut. It should prove interesting to determine whether these results are further confirmed in future. Currently, our group is examining whether a similar expression of MMP-8 can be detected from the CSF collected on filter papers, since such samples were collected during the clinical study in Angola. Moreover, we are planning to analyse how the CSF MMP-8 levels differ between different kinds of CNS infections, and if MMP-8 can be used in the differential diagnostics of bacterial meningitis.

7 CONCLUSIONS

The objectives of this thesis were to evaluate the benefit of a continuous β-lactam infusion combined with oral paracetamol as treatment for childhood bacterial meningitis in Angola, to examine the expression and the potential prognostic value of several inflammatory mediators in the CSF during meningitis and to study the impact of a child's vitamin D status on disease outcomes. The following conclusions can be made based on the respective studies:

I) The use of a continuous β-lactam infusion combined with high-dose oral paracetamol did not improve childhood bacterial meningitis outcomes in Angola when compared to conventional treatment.

II) MMP-8, MMP-9, TIMP-1 and MPO were all expressed in the CSF of children with bacterial meningitis. These inflammatory mediators associated with each other, reflecting the complex regulatory network of the inflammatory reaction. MMP-8 emerged as a promising marker of disease outcomes, particularly death.

III) Cathelicidin was expressed in the CSF of children with bacterial meningitis and reflected the extent of local inflammation. A decreasing CSF cathelicidin level during treatment predicted a better disease outcome.

IV) CSF cathelicidin closely associated with the bacterial genome count in the CSF. A high ratio of CSF cathelicidin-to-bacterial genome count predicted favourable outcomes, particularly in terms of hearing impairment.

V) S-25-OHD levels did not associate with the CSF cathelicidin concentration and did not predict mortality or severe neurological impairment in children with bacterial meningitis.

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